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Polymeric Biomaterials: Diverse Functions Enabled by Advances in Macromolecular Chemistry

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Abstract

Biomaterials have been extensively used to leverage beneficial outcomes in various therapeutic applications, such as providing spatial and temporal control over the release of therapeutic agents in drug delivery as well as engineering functional tissues and promoting the healing process in tissue engineering and regenerative medicine. This perspective presents important milestones in the development of polymeric biomaterials with defined structures and properties. Contemporary studies of biomaterial design have been reviewed with focus on constructing materials with controlled structure, dynamic functionality, and biological complexity. Examples of these polymeric biomaterials enabled by advanced synthetic methodologies, dynamic chemistry/assembly strategies, and modulated cell-material interactions have been highlighted. As the field of polymeric biomaterials continues to evolve with increased sophistication, current challenges and future directions for the design and translation of these materials are also summarized.

1. INTRODUCTION

The central role of polymers in the development of functional biomaterials has been fueled in large part by advances in synthetic methodologies that have enabled the production of well-defined and functionalized polymers that are responsive to desired physiological processes. Commodity synthetic polymers such as poly(hydroxyethyl methacrylate) (PHEMA), poly(lactic-co-glycolic) acid (PLGA), polyvinyl alcohol (PVA), and poly(ethylene glycol) (PEG) have been used widely and for many decades as contact lens and intraocular lens materials, formulated into thin films and microspheres as drug delivery

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reservoirs, and employed in the preparation of cell-compatible polymer scaffolds for tissue engineering.[1]

With the extensive development of living and controlled polymerizations, additional and numerous types of biomaterials have emerged with increasing levels of sophistication in the ability to tune and manipulate complex physical and biological properties. Ever-increasing functional group tolerance of controlled polymerization methods have enabled a large scope of modifications of polymer behavior (e.g., degradable constituents and biochemical moieties), as well as great flexibility of properties under a wide range of use conditions (e.g., pH, ionic strength, and chemical compounds).[2, 3] The development of recombinant methods as a tool in polymer science has complemented these advances in synthetic methods, and has significantly expanded the library of polymers containing sequences from naturally occurring proteins as well as components of native extracellular matrix (ECM), yielding biomaterials with tailored mechanical and cell signaling functions that mimic the complexity of native tissues.[4–9]

Taken together, the progress of the macromolecules community over the past five decades has not only enabled the development of functional biomaterials and novel medical products, but also the investigation and understanding of fundamental biological processes that underpin new approaches in medicine. Major contributions have been made in the well-controlled manipulation of materials structures over multiple lengthscales, the introduction of dynamically versatile modifications to introduce complexity that can both mimic and affect *in vivo* cell-materials interactions, and the production of sequentially programmed biomaterial systems for targeted delivery of drugs, genes, and cells *via de novo* stimuli-responsive strategies.

This perspective presents important milestones in the development of polymeric materials with defined structures and properties in order to highlight how this control has made, and will continue to make, key contributions to advancing the impact of biomaterials. Contemporary studies that focus on constructing materials with controlled structure, dynamic functionality, and biological complexity will yield new macromolecular approaches that foster prescribed interactions with biological systems. This will have significant benefit in the treatment of various diseases with small-molecule, macromolecule, and cell-based therapies, as well as in the regeneration of tissue and function after injury or disease.

2. TAILORED STRUCTURE AND FUNCTION

The design of polymeric biomaterials, complemented by an increased understanding of native tissue architecture and cell–material interactions, has evolved across lengthscales ranging from the molecular to the macroscopic. The advent of advanced synthetic polymer chemistry and recombinant protein expression techniques has provided exquisite control over the chemical composition and molecular architectures of materials that are key for their use as multivalent ligands, selective imaging agents, drug delivery vehicles and cell culture scaffolds. The development of orthogonal chemistries has afforded biocompatible preparation conditions and precise spatiotemporal manipulation of materials properties, permitting facile synthesis of biomaterials in the presence of cells and capturing some of the

inhomogeneity of native tissue. This section will highlight the advances in a toolbox of precision synthetic methods that have afforded polymeric materials with well-defined structures and tailored functions.

2.1 Advanced Synthesis and Conjugation Methods for Generating Functional Biomaterials

The development of controlled polymerization methods, covered extensively in other contributions to this issue, has yielded a plethora of new materials to tailor and/or understand biological responses. Advanced polymerization techniques, including living anionic polymerization, living ring-opening metathesis polymerization (ROMP), atom-transfer radical polymerization (ATRP), and reversible addition-fragmentation chain-transfer polymerization (RAFT), have been enormously valuable in applications ranging from drug sequestration to surface modification,[10–12] owing to their yield of polymers with predictable molecular masses, narrow molecular weight distributions, and high chain-end fidelity. Ring-opening metathesis polymerization of oxanorbornene-based systems has been extensively leveraged by Tew and co-workers to design cell-penetrating peptide mimics with well-defined structures for intracellular delivery via modulation of membrane interactions, cellular uptake efficiencies, and siRNA delivery.[13] The high level of control in newly developed, sulfur-free RAFT emulsion polymerizations has facilitated the production of highly organized, sequence controlled multiblock copolymers, conferring substantial potential for molecular targeting, recognition and nanomedicine.[14] Controlled polymerization strategies have also offered exceptional versatility for tuning polymer composition and brush density, for tuning interfacial properties and modulating cell adhesion, spreading, proliferation, and differentiation.[15] Readers are directed to the contributions in this issue, as well as to other recent reviews,[16, 17] for more information on this topic. Furthermore, the high degree of chain-end fidelity and the commercial access to various functionalized initiators have allowed the facile creation of polymer-protein/peptide conjugates and novel degradable materials that are sensitive to stimuli including glutathione, temperature, and photo irradiation.[3] The development and use of such functional initiators and reactive polymers in the design of bioconjugates and degradable materials have been a topic of recent reviews.[18, 19]

Modern polymerization techniques have also expanded to the realm of biopolymers and biomimetics, to afford precision synthesis of biomaterials that can actively interface with biologically complex environments. Living/controlled polymerization strategies of α-amino acid-N-carboxyanhydrides (NCAs), pioneered by Deming and co-workers, have been exploited for the construction of synthetic polypeptides based on both natural and side-chain modified polypeptides with well-defined sequences and architectures that support, amongst a range of behaviors, the facile self-assembly of hydrogel scaffolds and vesicles via manipulation of intramolecular interactions or secondary structures.[20, 21] Copolypeptide hydrogels with tunable physical properties (e.g., stiffness, porosity, and media stability) have been developed via the incorporation of α-helical copolypeptide domains, permitting prolonged release of both hydrophilic and hydrophobic molecules as well as facile preparation of cell suspensions for cell delivery.[22, 23] The polymerization of non-natural amino acid and side-chain modified NCAs (e.g., saccharide-, alkyne-, azido-modified monomers) has provided exceptional diversity in polypeptide functionality to both mimic

protein functions and interact with cellular systems.[24, 25] These versatile synthetic strategies have offered an economical and expedient approach for the production of high molecular weight polypeptides with precise structures and custom-made functionalities. Perhaps one of the most transformational advances in macromolecular approaches for biomedical applications has been the development of orthogonal chemistries of exceptional efficiency and functional group versatility, which have been almost universally applied for the modular design of sophisticated polymers and polymer conjugates with high levels of precision and control.[26–29] The well-known alkyne-azide cycloaddition (including copper-catalyzed alkyne–azide cycloaddition and the later developed strain-promoted azide-alkyne cycloaddition), Diels-Alder reaction, radical mediated thiol-ene chemistry, Michael-type addition, and hydrazone/oxime chemistry, have all been extensively explored.[30–32] The combination of high yield and outstanding selectivity afforded by these reactions has allowed the simple fabrication and functionalization of biomaterials, importantly under conditions sufficiently mild that they are regularly employed in the presence of biological molecules and living cells, as well as in the post-modification of cell culture matrices to mimic native biological structures.

Introduced by the groups of Sharpless and of Meldal,[33, 34] the copper(I)-catalyzed azide-alkyne cycloadditions (CuAAC, Figure 1A) has emerged as a popular methodology for efficient bioconjugation and facile production of biomaterials (e.g., well-defined hydrogel networks with reduced swelling).[35] To address issues with copper toxicity in biological systems, copper-free strain-promoted azide–alkyne cycloaddition (SPAAC, Figure 1B) reactions, pioneered by Bertozzi and co-workers, have driven the development of a series of cell-laden materials including hydrogel scaffolds and microgels in physiologically relevant conditions, allowing for the direct observation and thorough understanding of cellular processes including adhesion, proliferation and differentiation in three dimensions.[36, 37] Similarly, the recently developed tetrazine ligation (inverse-electron demand Diels-Alder reaction of tetrazine and trans-cyclooctene, Figure 1C), has also emerged as an important tool for the assembly of complex biomaterials under dilute conditions (e.g., hydrogel microspheres and channels,[38] polymer microfibers with cell attachment and alignment functions,[39] and homodimeric protein-polymer conjugates[40]), owing to its exceptionally fast reaction rates in the absence of any catalysts.

Given the crucial role of spatial and temporal control when producing biomaterials for cell encapsulation and tissue engineering, radical-mediated thiol-ene reaction (Figure 1D) has been extensively utilized to generate cytocompatible networks and modulate biochemical and mechanical properties within the matrix, providing a versatile tool for the manipulation and study of cellular activity in three dimensions.[41, 42] Recently, catalysis of the CuAAC reaction via the photochemical reduction of Cu(II) to Cu(I) (Figure 1E) has also been explored for fabricating patterned materials and patterned chemical modifications, expanding the toolbox of photo-mediated click reactions.[43–45] Similarly, the strategic combination of photolabile *o*-nitrobenzyl (*o*-NB) and coumarin derivatives as protecting groups for photocaged amine catalysts of thiol Michael-type additions has permitted photo-initiated surface patterning and formation of homogeneous networks with precise spatiotemporal control.[46–48] These photolabile groups can also be used as thiol protecting groups (Figure 1F), to provide spatiotemporal control over the patterning of 3D matrices

with biomolecules to guide cell attachment and function[49–51] as well as the production of hydrogel nanoparticles within nanotemplates.[52] The translation of these orthogonal chemistries to biomedical applications is a rich area for the design of sophisticated biomaterials with pristine definition and architecture at multiple lengthscales. Interested readers are also referred to reviews by Azagarsamy *et al.*[53] and Nimmo *et al.*[54]

2.2 Recombinant polypeptides

2.2.1 ECM-mimetic materials—With the monumental progress in the production of defined polymeric materials for biomedical applications, the design of protein-based biopolymers that capture biological functions has remained a powerful approach for generating biomaterials. Isolated ECM components such as Matrigel, collagen and fibrin have been extensively adopted but suffer from a lack of scaffold tunability, complicated bio/chemical structures, batch-to-batch inconsistency and potential immunogenicity.[55, 56] In addition, the materials properties and biological function of these types of matrices are intertwined, posing difficulties in the customization of the material or the study of a specific fundamental facet of cell-material interactions. The now routine application of recombinant technology to biopolymer design and production has fostered the development of modular biomaterials with monodispersity in molecular weights and site-specific and addressable biochemical compositions.[57, 58] The recombinant methods allow combination of desired structural properties with biofunctional moieties to create responsive microenvironments for studying *in vitro* cell-materials interactions as well as *in vivo* cell-material-tissue responses, with wide application, including in wound healing, angiogenesis, drug and cell delivery, and tissue regeneration (Figure 2).

While there has been excellent progress in the production of many polypeptides that mimic structural proteins such as silk, collagen, and elastin,[58–63] one of the most commonly employed recombinant polypeptides in biomaterials investigations have been elastin- and tropoelastin-based materials based on elastin's pentapeptide sequence VPGXG (where X can be any residue except proline). There have been myriad polypeptides produced with differences in protein sequence, amino acid composition, molecular length, architecture and hydrophilic-hydrophobic ratio;[64] these can be crosslinked *via* both physical and a variety of chemical methods, including transglutaminase-triggered enzymatic catalysis,[65] glutaraldehyde amine reaction, UV-initiated radical polymerization,[66] NHS-ester bis(sulfosuccinimidyl) suberate (BS3) crosslinking,[67] orthogonal alkyne-azide cycloaddition click chemistry[68, 69] and hydroxymethylphosphine-based Mannich-type condensation reaction.[70] These chemical crosslinking reactions have permitted fabrication of a wide spectrum of elastin-based materials ranging from hydrogels, films, particles, fibers, surface coating and porous scaffolds with tunable mechanical stiffness (Young's modulus in the range of 0.1~0.9MPa), uniaxial extensibility, stress relaxation properties, and resilience values,[65–67, 71, 72] although requiring chemical modification of the polypeptide, which can potentially alter protein function, reduce yields, and compromise monodispersity. Residue-specific incorporation of non-canonical amino acids[73–75] with new chemical functionality has afforded opportunities for highly-chemoselective, orthogonal *in vivo* protein coupling and labeling through reactions such as Staudinger ligation, hydrazide coupling, copper-catalyzed click chemistry, iodine/zinc-mediated backbone cleavage, and benzophenone-

initiated photo-crosslinking.[75–77] ELPs have also been modified with the non-canonical photoactive *para*-azidophenylalanine (pN$_3$Phe) to generate mechanically tunable "photoresist" materials without post translational chemical modification, enabling novel approaches for patterning of mechanical properties, manipulation of geometric shape and topography, and modulation of ligand receptor interactions.[77]

An emphasis on designing materials that are cell-instructive and matrix-interactive has cemented the value of recombinant polypeptides in biomaterials investigations. From the earliest work in which the now common fibronectin III-derived arginine-glycine-aspartic acid (RGD) integrin binding motif was incorporated into biosynthetic ECM scaffolds to promote cell adhesion and spreading,[78] current approaches employ a wide array of diverse, ECM-derived peptide modules. Guided by this concept, ELP-based scaffolds have been produced for neuronal tissue engineering, with the addition of multiple biological modules in otherwise identical material compositions, allowing for independent modulation of elastic modulus, matrix degradation kinetics, and cell adhesion.[67]

There also has been a recent resurgence in studies of the insect structural protein resilin,[79] which is found in nature in the joints and sound-producing organs of insects and has long been known for its unique mechanical properties (*e.g.*, large reversible extensibility, superior resilience, fatigue resistance and energy storage capability).[80] The production of the first recombinant resilins in 2005[81] ushered in a suite of resilin-like polypeptide (RLP) materials based mainly upon two putative motifs (GGRPSDSYGAPGGGN derived from *Drosophila melanogaster* and AQTPSSQYGAP adopted from *Anopheles gambiae*) with tailorable compositions, crosslinking chemistries, and material properties that largely capture the excellent resilience of the native protein.[82–84] These encouraging findings have initiated applications of RLP materials in biorubbers, nanosprings, diagnostic biosensors and in regenerative medicine as elastomeric tissue engineering scaffolds.[81, 84–87] Our group has designed constructs combining the resilin consensus motif (GGRPSDSYGAPGGGN) with bioactive domains to impart cell adhesion, proteolytic degradation and heparin immobilization functions in tunable bioelastomers for mechanically demanding applications such as in therapies for vocal folds and cardiovascular tissues.[83, 88–90] Hydrogels with different cell adhesion morphologies and matrix degradation profiles can be produced without compromising desired mechanical features (e.g., oscillatory shear moduli, Young's moduli, resilience and stress relaxation),[84, 91] offering substantial opportunities for reproducibility, tunable biocomplexity and dynamic multifunctionality (Figure 3).

2.2.2 Assembly and Delivery—In addition to uses in ECM-mimetic materials, the unique and quantitatively tunable inverse transition behavior[92] of the ELPs (and some RLPs) has motivated widespread use of these polypeptides in the responsive assembly of nanoparticles (Figure 4A), which offer opportunities not only for sequestration of drugs but also for triggering delivery on the basis of multivalent interactions. Myriad thermally responsive ELPs (with T_t values easily tuned with polypeptide concentration, molecular weight, guest residues, pH, ionic strength, salt, ligands[93], and chemoselective alkylation of methionine,[94] Figure 4B), have been produced for biomedical applications including gene/drug delivery,[95] tissue engineering[96] and emerging optoelectronic devices for bioimaging purposes.[97] Most of these applications are largely based on hyperthermic treatments that

trigger the coacervation of the ELPs into nanoparticles or hydrogels and allow their localization to tumor (Figure 4A),[98, 99] although preformed drug-loaded ELP-based micelles can be programmed to disassemble and release cargo molecules upon encountering the tumor environment.[100] A calcium-binding peptide from calmodulin has been introduced and periodically interspersed within an ELP to generate novel protein polymers with both thermal and calcium sensitivities to trigger well-controlled assembly.[101] Such fusion of small, biologically active peptides to ELPs has also been investigated for producing new ELPs for other biomedical applications.[102] ELPs fused to the αB-crystallin peptide (which protects human retinal pigment epithelial (RPE) cells from oxidative stress during the progression of age-related macular degeneration (AMD)), modulated the assembly and intracellular uptake pathway of the native αB-crystallin peptide.[103]

The production of large libraries of these polypeptides has been enabled by the optimization of robust, high-throughput gene synthesis methods (e.g., overlap extension rolling circle amplification (OERCA) and codon-scrambling algorithm-implemented PCR-based gene synthesis);[104, 105] these high-throughput methods have identified a range of polypeptides that are sensitive to multiple stimuli. Very recent studies by the Chilkoti group have presented general sequence heuristics to predict phase transitions across all intrinsically disordered proteins (IDPs) comprising structure-breaking residues Gly and Pro;[106] these guiding principles can enable sequence-level design of predictable LCST, UCST or dual phase-transition behaviors in IDPs. They also allow probing of phase separation phenomena at the proteome level, with applications in exploiting protein phase transitions for biomaterials and understanding their role in homeostasis and disease.

The Rec1-resilins, as well as several other RLPs, exhibit intriguing dual phase-transition behavior characterized by both reversible LCST and UCST,[107, 108] which has been employed to tailor transition temperature and sizes of RLP-based nanoparticles (Figure 4C),[109] as well as in protein purification, functionalization of surfaces, and immobilization of drugs, nanoparticles, enzymes and catalysts for delivery and diagnostic applications.[110, 111] Our group has also demonstrated that the phase separation behavior of RLP can be manipulated by the addition of poly(ethylene glycol), to yield well-defined microstructured hydrogels (Figure 4D) for applications ranging from mechanical reinforcement to regenerative medicine.[112] The well-defined temperature- and pH-sensitive responses of selectively engineered RLPs and ELPs suggests the promise of these recombinant polypeptides across a range of applications.

2.2.3 Biorecognition and Adaptable Networks—While the majority of selected structural and bioactive peptide domains have been inspired from native ECM proteins, peptide sequences from non-ECM proteins have also been repurposed from their physiological function in the generation of responsive biomaterials.[113] Pioneering examples from the Tirrell laboratories adapted coiled-coil motifs as physical crosslinks in self-assembling protein biomaterials;[114, 115] hydrogel gelation mediated by the association of the coiled-coils can be triggered by external stimuli and the erosion kinetics modulated by selective molecular recognition and orientational discrimination of select coiled-coil domains.[116] The resulting injectable hydrogels exhibit typical shear thinning and rapid recovery after large deformation, with properties suitable for *in vivo* cell delivery. More

recently, conjugation of thermoresponsive synthetic polymers to these polypeptides has provided an additional handle to improve mechanical properties.[115]

Similar types of behavior have also been recently for a triblock protein comprising a midblock derived from the N-terminal fragment of the rat cartilage oligomeric matrix protein (COMP, which adopts a homopentameric coiled coil conformation) flanked by two ELP end-blocks (Figure 5A).[117] Such interactions, in combination with biomolecular recognition, can also be applied to reversibly tune the accessibility of immobilized adhesive ligands, mimicking the *in vivo* temporal regulation of ECM-anchored signaling ligands, with applications in guiding cellular behavior during development.[118] Computationally predicted peptide domains have also been similarly valuable in supporting hydrogel formation. Hetero-assembling polypeptides comprising a WW domain (an anti-parallel, triple-stranded β-sheet peptide fold) that specifically recognizes proline-rich sequences encoded in a second polypeptide, support the formation of a reversible network upon simple mixing (Figure 5B).[119] The reversibly shear-thinning MITCH hydrogels supported *in vitro* growth and differentiation of neural stem cells and retained *in vivo* survival of injected adipose-derived stem cells in a subcutaneous mouse model, demonstrating the use of these approaches for encapsulating cells or any sensitive biological cargo for therapeutic delivery.[120]

These approaches have also proven valuable for the production of spontaneously crosslinking hydrogels. The recently discovered SpyTag and SpyCatcher reactive protein pair, derived from the fibronectin-binding protein (FbaB) of *Streptococcus pyogenes*, provides molecularly encoded “orthogonal chemistry” that has been exploited by the Tirrell group to produce spontaneously formed covalent linkages between ELPs under physiological conditions.[121] The resulting “network of spies” comprised cell-adhesion ligands, matrix metalloproteinase-1 cleavage sites and full-length globular proteins (mCherry and leukemia inhibitory factor (LIF)), and encapsulated mouse embryonic stem cells remained pluripotent without the additional introduction of LIF, demonstrating the utility of these genetically coded, covalently reactive modular protein pairs for producing information-rich biomaterials that can direct stem cell responses.[122]

The progression of recombinant technologies coupled with bottom-up design strategies has enabled the fabrication of a variety of biomaterials with independent control of functional modules, mechanical features and biochemical signals, offering great potential as synthetic ECM scaffolds to investigate cell-microenvironment interactions. Recently developed powerful alternatives such as predictive computational modeling and high-throughput combinatorial screening have also been employed to create complex and multifunctional matrices comprising sequences beyond the library of naturally evolved-domains and endowed with *de novo* designed functionalities. This suite of approaches and protein-based materials are suitable for both fundamental biological studies and translational development for clinical applications.

3. CONTROL OVER DYNAMIC FUNCTIONALITY

Dynamic materials with spatially or temporally varied physicochemical properties have revolutionized biomaterials design and utility in studying cell-material interactions. A wide

variety of chemically labile bonds have been exploited as degradable linkages to endow biomaterials with dynamic features. Such design has evolved from simply achieving clearance of the materials via passive ester hydrolysis to introducing specific temporal control over properties in order to manipulate the release of cargo as well to engineer spatiotemporal changes in matrix properties relevant for tissue constructs.[123124] The ability to tune properties spatiotemporally is essential for mimicking the dynamic complexity of the native cellular environment and understanding *in vivo* cell behaviors.

3.1 Programmed Degradation

Temporal changes in network degradation have commonly been achieved via simple hydrolysis or cell-mediated proteolysis. Historically the most common approach, the inclusion of hydrolytically degradable components in hydrogels allows manipulation of molecule release to local cells and tissues as well as control of distribution of ECM molecules secreted by encapsulated cells.[125, 126] For example, hydrolytically degradable hydrogels have been engineered with different degradation profiles to control the spatial distribution of ECM components for formation of neocartilage.[125, 127] However, hydrolysis usually occurs at pre-programmed rate throughout the bulk of a material, which often does not mimic the rate of matrix remodeling *in vivo*.[128, 129]

In order to address the need for cell-mediated modification of biomaterials, peptide sequences that can be cleaved by cell-produced proteases, such as matrix metalloproteinases (MMPs), are now routinely incorporated into hydrogel crosslinks. PEG-peptide hydrogels, pioneered by Hubbell and co-workers and employed creatively by many research groups, include protease-degradable crosslinks to engineer tissue constructs, through the incorporation of growth factors released via cellular cues and morphogenesis of encapsulated cells into a variety of tissue structures (such as bone and vasculature).[130, 131] With the increasing understanding and growing appreciation of the unique biological and physicochemical properties of glycosaminoglycans (GAGs) and polypeptides,[79, 132] protease-degradable GAG (e.g., heparin and hyaluronic acid)[133] and polypeptide and protein hydrogels (e.g, ELPs, RLPs, and others)[83, 84, 134] have also been produced to contain MMP-sensitive peptide crosslinkers/sequences. Another appealing advantage of the protease-sensitive hydrogels is that the rate of their degradation can be selectively modulated in pathologies where protease activity is altered, such as rheumatoid arthritis, cancer, and after myocardial infarction.[124] MMP-sensitive hydrogels that locally release a recombinant tissue inhibitor of MMPs (rTIMP-3) in response to MMP activity, for example, have been explored to afford on-demand MMP inhibition after myocardial infarction, where the drug release rate and dose were controlled through a feedback mechanism.[135]

3.2 Stimuli-Responsive Materials

In addition to programmable degradation via the introduction of hydrolytically or enzymatically cleavable linkages, biomaterials that can respond to specific environmental stimuli have become a mainstay approach for obtaining on-demand, tailored release profiles of therapeutics and cells. A wide range of stimuli, either exogenous (variations in temperature, magnetic field, ultrasound intensity, light or electric pulses) or endogenous (changes in pH, enzyme concentration or redox gradients), have been extensively explored

for the implementation of such responsive systems.[136, 137] Due to the universal appreciation of pH differences between specific organs (e.g., the gastrointestinal tract), intracellular compartments (such as endosomes or lysosomes), and pathological situations (such as cancer), pH-responsive materials that can undergo protonation or cleavage reactions in response to environmental pH variation have been historically exploited to develop gastric-resident devices in prolonged oral drug delivery, to offer intracellular delivery of payload, and to provide tumor-targeted release and increased tumor retention.[138, 139]

Thermoresponsive materials have been perhaps the most extensively studied, and there are myriad reports of the use of poly(N-isopropylacrylamide) (PNIPAM) in biomedical applications aimed at controlling enzyme activity, cell adhesion, and drug release.[140] With the recent progress in peptide synthesis and protein expression (see above), however, peptides and recombinant proteins have also emerged as attractive building blocks for the construction of thermoresponsive materials. The unfolding transition of the peptide domains can be finely tuned, as well as engineered to be responsive to specific ligands or use conditions. Temperature-sensitive, coiled-coil peptides that unfold and dissociate above their melting temperature (~40°C) have been inserted in the membrane of liposomes to optimize drug release under mild hyperthermic conditions.[141] Shear-thinning protein hydrogels utilizing the self-assembly of PNIPAM polymer-peptide conjugates have been developed to introduce thermoresponsive reinforcement of the physical crosslinked network, which can significantly retard material biodegradation and prolong transplanted cell retention time after *in vivo* injection.[115, 142] Elastin-like polypeptides (ELPs) that exhibit LCST-like phase transition behavior, as introduced above, have also been utilized to engineer delivery vehicles for the hyperthermia-assisted delivery of various therapeutics including peptide and small molecule drugs.[95] Collagen-like-peptides (CLPs) that form triple-helix crosslinks have been exploited to formulate injectable hydrogels with thermo-reversible gelation behaviors.[143, 144] Our group has recently introduced thermoresponsive nanostructures based on the self-assembly of elastin-*b*-collagen-like peptide bioconjugates (Figure 6A), where facile formation of well-defined vesicles was enabled at physiological temperature and the resolubilization of the vesicles was achieved at elevated temperatures upon unfolding of the CLP domain.[145] The precision synthesis of these materials, coupled with known structure-property relationships of specific amino acid sequences, permits simple variations in the relative lengths and sequences of the peptide/polypeptides, which can be used to tailor the thermoresponsive behavior of these systems and impart triggered assembly/disassembly within physiologically and clinically relevant temperature ranges.

Beyond changes in environmental pH and temperature, levels of endogenous thiol-containing molecules such as glutathione (GSH), an antioxidant localized to intracellular compartments and often overproduced in tumor microenvironments,[148] have been a target of particular interest for stimuli-responsive materials. The incorporation of GSH-sensitive linkages in biomaterials can permit selective degradation in the presence of GSH and allow the targeted and triggered delivery of therapeutic molecules relevant to cancer applications. As a prevailing strategy, disulfide linkages that can undergo cleavage upon exposure to GSH have been commonly incorporated into biomaterials via oxidation, disulfide-containing crosslinkers, and thiol-disulfide exchange reaction, allowing the construction of GSH-sensitive nanocarriers (e.g., micelles, polymersomes, and nanogels) and hydrogels.[149, 150]

These materials maintain excellent stability in circulation and in extracellular fluids, and undergo rapid degradation inside cells/tumor microenvironments. However, the rapid cleavage kinetics of disulfide bonds (half-lives ranging from 8 to 45 min)[151] temporally limits the delivery process (ca. 12–24h). Alternatively, novel GSH-sensitive hydrogels developed by our group, in which degradation is mediated by retro Michael-type addition and subsequent thiol exchange (Figure 6B), have demonstrated increased stability against GSH with 10-fold slower rates of degradation.[146, 152] which can offer selectivity as well as more extended delivery. The use of nanoparticle-crosslinked, multicomponent hydrogel systems may further extend the lifetime of related hydrogels, owing to the steric hindrance and local hydrophobic environment of the arylthioether succinimide crosslinks at the polymer-nanoparticle interface.[153] The enhanced stability permits release of encapsulated cargo molecules over longer timescales (ca. 3–6 days),[153, 154] demonstrating significant promise for tailoring therapeutic release within tumor microenvironments.

In addition to endogenous glutathione, other biological stimuli based on specific signal biomolecules have also been a focus of study in the design of biomaterials that undergo responsive assembly and disassembly as well as dynamic volume and shape changes. Conformationally dynamic proteins that can undergo hinge motion upon binding of specific biochemical ligands (e.g., glucose, ATP) have been incorporated into hydrogel networks to induce reversible volume changes.[155] In a notable example demonstrated by Murphy and co-workers, calmodulin-containing hydrogels underwent a significant volume decrease in the presence of the drug trifluoperazine, which shifted the conformation of calmodulin from an extended dumbbell shape to a collapsed globular conformation.[156] Further, dynamic hydrogels have also been developed based on competitive biomolecule interactions (such as antigen-antibody binding and saccharin-lectin binding) to introduce reversible swelling/shrinking behaviors in response to target biomolecules (e.g., antigens and tumor-specific marker glycoproteins) for potential applications in molecular diagnostics.[157, 158] Additionally, heparin-protein interactions have also been exploited by our group as reversible crosslinks in the design of cell surface receptor-responsive hydrogels. Taking advantage of the receptor-mediated targeting of growth factors to growth factor receptors, the hydrogels demonstrate receptor-mediated gel erosion and the cell-responsive, sustained release of vascular endothelial growth factor.[159, 160] Similarly, thrombin-cleavable heparin hydrogels have been explored to introduce feedback-controlled regulation of heparin release, enabling the homeostatic control of blood coagulation activation in living tissues.[161] The use of these dynamic mechanisms, including allosteric protein conformational changes and competitive ligand-protein binding, suggests a promising strategy in the design of bioresponsive materials to create spatially patterned actuators, tunable biosensors, dynamic growth-factor delivery systems as well as anticoagulant coating medical devices.

Owing to the non-invasive nature and remote spatiotemporal control of light, the development of photo-induced methods of controlling materials properties has represented a major innovation in the use of polymers in biomedical applications. Photodegradable systems incorporating photolabile *o*-nitrobenzyl (*o*-NB) and coumarin derivatives (Figure 6C) allow real-time manipulation of the physical or chemical properties of materials,[162] offering opportunities to spatiotemporally pattern biological signals within a hydrogel matrix, to capture the complex signaling cascades found in nature, as well as to tailor

patient-specific drug delivery and therapeutic regimens. Studies pioneered by the Anseth group, and expanded by Kloxin, Kasko, and others have introduced photodegradable hydrogels with photo-cleavable crosslinks for controlling network structure (e.g., mechanical stiffness and shaped features) as well as on-demand release of pendant functional groups,[147, 163, 164] allowing the temporal and spatial regulation of desired cell functions and an improved understanding of cellular responses in heterogeneous physical environments.[165] Incorporation of *o*-nitrobenzyl derivatives into nanoparticles and polymer assemblies has yielded photoactivatable, acidifying nanoparticles for controlled acidification of impaired lysosomes[166] as well as light-sensitive cationic nanocarriers for nucleic acid delivery and enhanced spatiotemporal control of gene activation.[167, 168] Photo-controlled radical polymerization facilitated by the use of UV-light-responsive trithiocarbonate (TTC) “iniferters” has also been explored to provide a strategy for altering the structure and composition of covalent polymer gels.[169] Gels formed by NIPAM polymers possess TTC moieties at the center of each network chain can undergo a photo-growth process upon light exposure, leading to an increase in the average MW between crosslinks via direct extension of network chains and providing access to soft materials with both mechanical and chemical 3D gradients. Similarly, the photoisomerization of azobenzene has been leveraged to modulate the matrix elasticity in a reversible manner.[170] Azobenzene can be incorporated into peptide crosslinkers of PEG hydrogels to reversibly stiffen and soften the networks upon stimulus with light, enabling investigations of the effect of dynamic changes in matrix stiffness on the behavior of adhered cells. Photo-sensitive methods have also been useful for the staged and sequential delivery of different stem cell populations, with opportunities in generating complex tissues that require multiple cell types.[171] The incorporation of multiple photolabile moieties with wavelength-specific cleavage kinetics allows wavelength-controlled release of multiple growth factors/molecules in a sequential and multistage fashion, which may better mimic the temporal profiles and spatial gradients of the healing process for improved tissue regeneration.[172, 173] Recently, photolabile moieties have also been integrated with GSH-sensitive linkages by the Kloxin and Kiick groups, to generate multimodal degradable hydrogels that respond to both externally applied light and reducing microenvironments, creating complex degradation profiles and therapeutic regimens as necessitated by the specific biomedical application of interest.[174] Limitations owing to the high energy and short penetration depth of UV light through biological tissue have motivated the synthesis of photolabile moieties susceptible to biologically benign near-infrared (NIR) via two-photon absorption.[175, 176] Incorporation of biocompatible up converting nanoparticles (UCNPs)[177–179] will expand the use of photoresponsive biomaterials, allowing for longer-wavelength irradiation with deeper tissue penetration, lower scattering, and minimal harm to tissues.

4. CONTROL OVER BIOCOMPLEXITY

Advances in dynamic and responsive materials, like those described in previous sections, have enabled the engineering of materials that are able to respond to, and integrate with, biologically complex environments. Insight into how information is processed and exchanged in tissues and organ systems has provided new opportunities to probe cell-material interactions. Given difficulties encountered in capturing multiple biological

functions in a single material during its synthesis, recent approaches have focused instead on reducing the biological complexity into essential elements and then developing materials able to perform specific and select functions. Peptide, polypeptide, and bioconjugate materials have afforded materials able to interact with the biological environment and alter cell function accordingly.

4.1 Cell Sorting

Given the precision that can be exerted over polymeric substrate geometry and patterning, physical isolation of cells on the basis of size or deformation under pressure has been achieved using UV- and temperature-sensitive polymers.[180] However, limitations in detecting subtle physical and biological differences between cell types have required approaches that utilize specific, affinity-based interactions between materials and cell-surface receptors for cell isolation. Conjugation of linear PEG, or amphiphilic triblock polymers (PBA/PEA/PMA), to targeting ligands specific for circulating tumor cells (CTC) have been deployed on the surfaces of nanoparticles, providing a mechanism for CTC detection *in vivo* or isolation from drawn blood specimens.[181, 182] Covalent conjugation of proteins, antigens, antibodies, and oligonucleotides to polymer backbones has also facilitated cell-surface receptor interactions with polymeric substrates,[183] and has been utilized in microfluidic devices to increase cell capture efficiency by altering the surface topography or by conjugating targeting ligands.[184, 185] While microfluidic approaches are promising, the high-throughput recovery of highly pure, isolated cells from microfluidic devices remains challenging. The development of (bio)polymers that are sensitive to phase-changes (temperature, UV-light, enzymes, Ca^{2+} depletion) may improve retrieval of cells from microfluidic channels.[184, 185]

Assembly and degradation chemistries commonly utilized in the construction of bulk hydrogel materials have also been applied to cell isolation strategies. Encapsulation of desired cell populations, for example, has been achieved by applying temporary polymeric coatings to target cells, which protect antigen-positive cells while unprotected cells are lysed (Figure 7).[186, 187] Photocleavage of *o*-NB linkages in the polymer then allows retrieval of highly purified cell populations post-lysis.[186] The sequential application of two photopolymerization steps to thiolene-based polymers has allowed the capture of a population of cells, followed by selective encapsulation of undesirable cells and retrieval of the desired cell population.[188] Polymer-based cell-sorting strategies have thus enabled both analysis of individual cell types and enrichment of specific cell populations for therapeutic use.

4.2 Stem Cell Therapies

Stem cell-based therapies are attractive approaches for promoting regeneration of tissues damaged by injury or disease. Unfortunately, these therapies have been plagued by challenges associated with survival of cells during injection and functional integration of transplanted cells, severely limiting clinical efficacy.[189] To address these issues, sophisticated polymeric systems that can protect transplanted cells from rapid death and simulate engraftment with the host tissue have been of significant value.[190] The use of weakly crosslinked injectable materials has been demonstrated to protect cells from

mechanical shear and extensional forces that arise during syringe needle flow.[142] Gelation kinetics must be carefully tuned in order to successfully deliver cell-laden matrices to intended locations without cell aggregation or unwanted gelation. Materials comprising dynamic covalent bonds or supramolecular assembly have represented a major advance for these uses.[191, 192] For example, utilization of associating protein motifs in mixing-induced two-component hydrogels (MITCH; Figure 5B) has yielded other injectable matrices that are cell protective.[142] Secondary crosslinking steps, such as those achieved using thermoresponsive polymers, have been shown to support long-term cell survival, sustain matrix integrity, and limit fast biodegradation that is observed in hydrogel systems that exhibit weak physical crosslinks.[142]

Transplanted cells face a multitude of challenges post-injection including lack of supportive matrix, hypoxia (oxygen limitation), and inflammatory responses, and materials that aid in cell survival for specific clinical applications are of great importance. For example, materials engineered to deliver oxygen directly[193] or deliver factors that aid in cell survival[194] are enormously useful for delivering cells to ischemic tissues such as those present following acute myocardial infarction. Beyond assisting cell survival, transplanted cells must also overcome the immune and inflammatory response that occurs in injured tissue. Polymeric carriers were initially designed to shield cells from this harsh environment; however, advanced synthetic methodologies have enabled the design of bioactive materials that can direct immuno-activation or suppression. While this topic will be further discussed in Section 4.4, the design of specific elements within materials, including the use immunomodulatory peptide self-assemblies, has greatly aided cell transplantation therapies.[195] Successful employment of biomaterial carriers also requires specific materials properties that enhance cell function once placed *in vivo*. Matrix mechanics, biochemical ligand presentation, delivery of soluble factors, and degradation profiles all largely influence cell differentiation, maturation, and secretion profiles.[196] It is important to note that no single formulation is optimal for all stem cell or tissue types; thus, continued research probing the relationships between biomaterial properties and stem cell function will remain of significant importance.

4.3 Directing Cell Phenotypes

Polymer synthetic advances have enabled the production of polymers with highly tailored properties that can be altered to guide cell behavior; the presentation of biochemical cues, matrix architecture, and mechanical properties can be optimized for specific therapeutic applications.[197] Small changes in the physicochemical properties of a polymer matrix, such as hydrophobicity and chemical functionality, can also influence cell phenotype and lineage commitment.[198] For example, hydrophobic polymers, which promote protein absorption and subsequent cell adhesion, have been shown to enhance cell attachment, proliferation, and differentiation.[198, 199] The incorporation of peptide and other bioactive ligands into both synthetic and biopolymers has also provided a broad range of functionalities, including cell adhesion,[84] growth factor sequestration,[200] and cell signaling;[201] simple incorporation of peptides, as well as alterations in gradients in the concentration of single and multiple peptides,[202] offer important strategies to enhance cell-matrix interactions and direct cell phenotype in wound healing, cancer, and vascular tissue engineering applications.

The vast array of chemical approaches developed for macromolecular design has further enabled the incorporation of growth factors, cytokines, DNA, RNAi, and therapeutic drugs within tissue engineered constructs to direct cell behavior and phenotype. Affinity-based delivery systems, such as those utilizing heparin or specific ligands to associate proteins with the network, have been designed to release various factors, ranging over days to weeks.[159, 203] Alternatively, cell-mediated release of covalently linked macromolecules (as mentioned above) can be regulated by conjugation of the growth factors to MMP-cleavable sequences in the polymer backbone, allowing for local therapeutic angiogenesis.[204] The use of bioorthogonal photoactive chemistries has further provided mechanisms to control the addition and removal of biochemical signals to control cell adhesion and motility,[205] promote endothelial tubulogenesis,[206] and initiate differentiation of stem cells.[6] While photochemical patterning may prove difficult to implement *in vivo*, the inclusion of multiple matrix molecules within scaffolds marks additional progress toward the sophisticated complexity that is present in native ECM.

Cell signaling is also regulated in large part by the micro- and nano-scale organization and structure of the matrix in which cells reside. The chemical versatility of contemporary polymerization methods has enabled inclusion of topographical features that mimic those present in the native ECM. The combination of chemical and peptide-based approaches has allowed thoughtful design of the molecular interactions and processing conditions that drive nanoscale architecture, secondary structure formation, and self-assembly of tubes, fibers, and scaffolds.[207] Fiber-like scaffolds, designed to mimic fibrillar ECM proteins, have been shown to regulate cell migration, proliferation, and epithelial-to-mesenchymal transition (Figure 8A).[208, 209] Multiple processing strategies have been applied to develop microstructured materials, including photopatterning[210] and selective degradation,[211] as mentioned above. Such approaches yield materials that exhibit improved cell attachment and migration,[212] as well as enhanced tissue infiltration into scaffolds.[213] Additionally, as mentioned above, microstructured, elastomeric hydrogels materials explored by our group via the liquid-liquid phase separation of protein-PEG solutions,[112] also present a simple approach to control the spatial organization of mechanical and biochemical cues that cells experience within 3D microenvironments.[214]

Protein and polymer organization and structure further play large roles in dictating mechanical properties, which profoundly influence and regulate the phenotypic behavior of cells. Pioneering work by the Discher laboratories established the key importance of substrate stiffness on stem cell differentiation and phenotype;[215] and many others have demonstrated further utility of biopolymers for delineating the effects of stiffness on the phenotype of various cell populations in both 2D and 3D *in vitro* models.[123] For example, neurite outgrowth from dorsal root ganglia was increased on compliant ELP hydrogels, as compared to stiffer gels.[216] We have also shown the implications of stiffness on a variety of vascular and hematopoetic stem cells (Figure 8B), demonstrating differential attachment, proliferation, and gene expression in response to matrix mechanics.[217, 218] Recently, Mooney and co-workers have also highlighted the importance of considering matrix stress relaxation in the design of biomaterials for cell culture, where alginate hydrogels with more rapid relaxation were shown to enhance cell spreading, proliferation and differentiation of mesenchymal stem cells (MSC) in 3D culture.[219]

The inclusion of stimuli-sensitive macromolecules within scaffolds has enabled dynamic control of mechanical properties, thus allowing study of how changes in ECM rigidity, such as those during development and disease pathogenesis, impact cell phenotype. As previously mentioned in Section 3, polymeric matrices containing photolabile polymers[220] and proteolytic peptide sequences[221] have provided mechanisms to study cell response and differentiation fates in response to temporal decreases in stiffness, as well as to microscale variations in the spatial patterning of matrix mechanics. Multifunctional polymeric systems with dual modes of crosslinking provide platforms, mediated through radical, temperature, or cationic methods, in which the impact of matrix stiffening on cell phenotype and function can be studied.[222, 223] However, determining the relationships between biomaterials properties and cell function remains convoluted, as manipulation of one parameter often results in unintended changes in several other biomaterials properties. Since cells respond to biomechanical and biochemical cues in a context-dependent manner, the development of materials with independent control over these cues will enable systematic parsing of cell-material interactions.

4.4 Immunomodulation Strategies

The immune system plays a critical and positive role in the integration of biomaterials. Thus, biomaterials design strategies have focused on the development of immunomodulatory biomaterials in order to control immune cell response and facilitate functional integration of polymeric implants or retention of drug delivery carriers. Similar to reported behaviors of somatic and stem cells, microenvironmental cues presented by biomaterials play a crucial role in modulating immune cell behavior.[224] Alterations in polymeric substrate stiffness, topography, and geometry have been shown to alter inflammatory cell response to polymeric implants,[224, 225] while the size, shape, and surface chemistry play a significant role in extracellular trafficking, immune cell recognition, and intracellular processing of particulate systems.[226] The inclusion of cell adhesion moieties and cell signaling molecules, as well as specific features of their presentation, have been shown to guide inflammatory cell recruitment and adhesion,[227] and can thus also largely impact the degree of immunomodulation of a polymeric material. For example, self-assembling fibrillar peptides alone have generally not raised inflammatory or immunogenic responses in animal models;[228, 229] however, fusion of the Q11 (QQKFQFQFEQQ) peptide to antigenic ovalbumin peptides formed multivalent fibrillar conformations able to provoke strong, long-lasting antibody responses (increased IgG1, IgG2a, IgG3, IgM) without the use of adjuvants.[230]

Biomaterials-based therapies have been designed for vaccine delivery and cell-based immunotherapy.[226] Vaccine efficacy has been improved with the use of synthetic delivery systems, including nanoparticles and thin films, to deliver vaccine subunits and corresponding molecular adjuvants to targeted lymphatic tissues.[231, 232] Polymeric particulate systems have further been utilized to boost anti-tumor immunity in cell-based immunotherapies. For example, adjuvant-containing nanoparticles coupled to cytotoxic T cells during adoptive cell therapy are able to regulate the molecular interactions in the T-cell synapse and prolong the function of tumor-specific cytotoxic (CD8+) T cells during cancer treatment.[233, 234] Similar strategies have been utilized to alter the fate of helper (CD4+) T

cells in order to promote tissue-graft survival.[235] Design strategies have also enabled researchers to engineer immune responses outside of lymphoid organs. For example, biomaterials functionalized with inflammatory cytokines, immune danger signals, and tumor lysates were able to control the localization, activation, and antigen loading of dendritic cells *in vivo*, and subsequently led to the priming of antitumor T cells.[236, 237] Accordingly, these examples demonstrate the substantial progress that biomaterials-based immunotherapies have made in regulating the immune response.

5. CONCLUSIONS AND FUTURE PERSPECTIVES

This perspective highlights the recent developments in the design of polymeric and (poly)peptidic biomaterials with a specific emphasis on advances in tailoring biomaterials structures and properties, introducing dynamic functionality, and capturing biocomplexity. The robustness and versatility of modern polymerization methods offer great flexibility in tuning macromolecular properties for desired biomedical applications, and the modularity and precision provided by peptide, polypeptide, and bioconjugate materials further provide versatility, control, and manipulation of physicochemical and biochemical properties. The limits of these synthetic strategies have certainly not yet been reached, and systematic investigations in reducing the cytotoxicity of polymers produced by controlled polymerization techniques will greatly expand the use of these well-defined synthetic materials in clinical applications.

Orthogonal and dynamic chemistries will continue to enable the production and functionalization of biomaterials that capture aspects of native biological structures. With increasing opportunities to better mimic the dynamic environment in tissues and organs, future studies on degradable and stimuli-responsive materials will require continued assessment of degradation and pharmacokinetic profiles in vivo, as well as development of an expanded toolbox of clinically relevant approaches that could offer patient-specific treatment regimens, such as, for example, polymer and nanoparticle platforms sensitive to tumor-specific ligands, receptors, and/or biologically benign wavelengths of light.

Parallel to these advances in synthetic chemistry tools that allow molecular control of biomaterials, the development of novel processing methods that impart well-defined macrostructure (e.g., phase separation and 3D printing), will also play a role in creation of complex materials with utility in biomedical applications such as enhanced cell and tissue infiltration. Additionally, the continued development of high throughput and combinatorial methods will offer key tools for biomaterials synthesis and deconstruction of the native cellular microenvironment as well as complex cell-material interactions.[238, 239] It will also be important that the future developments of these techniques are achieved in a manner that both allows manufacturing at large scales and reasonable cost, and also overcomes regulatory challenges with the translation of new materials.

With the continued convergence and synergies in tailoring structure, dynamic function, and biological complexity, the prospects are promising for biomaterial systems that allow exquisite structural assembly, enable active interactions and perform tailored, high-level functions at complex biological interfaces. Such function will enable the tuning of cell-

materials interactions that will be key for expanding our knowledge of the underlying mechanisms that control cell and tissue fate and that result in new and lasting contributions to the biomedical sciences.

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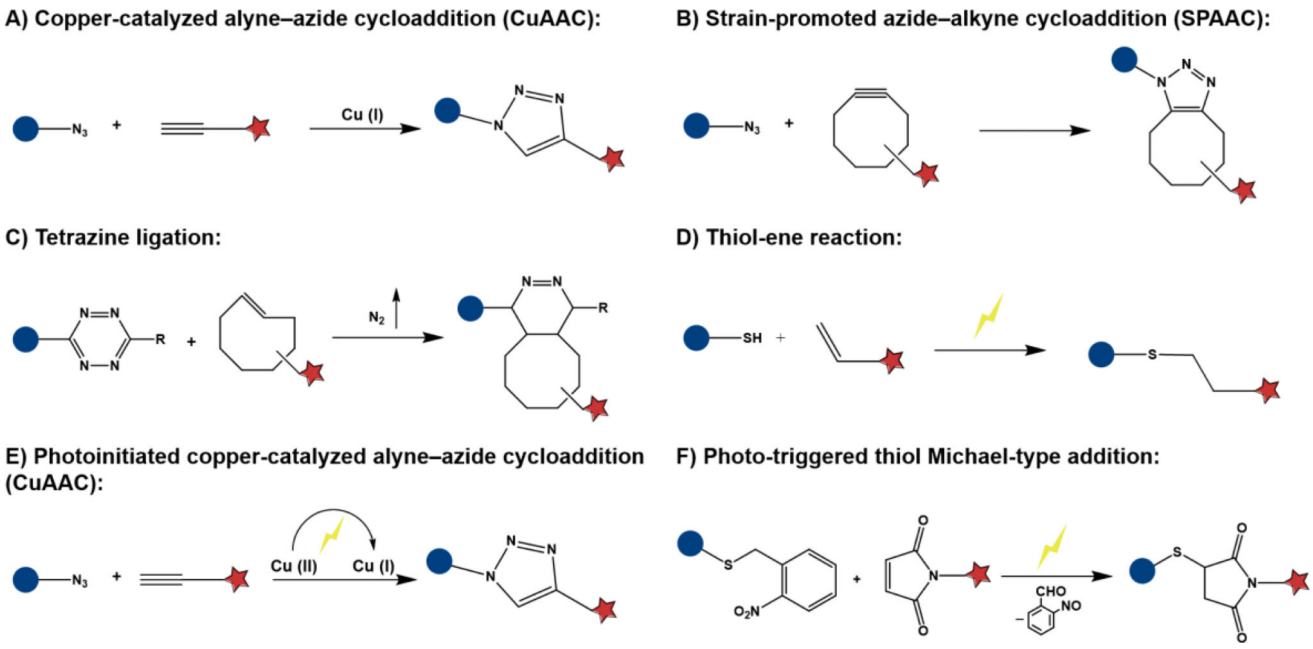

Figure 1.

Examples of orthogonal chemistries that are commonly used in the production of functional biomaterials. A) Copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC), B) Strain-promoted azide–alkyne cycloaddition (SPAAC), C) Tetrazine ligation, D) Thiol-ene reaction, E) Alkyne–azide cycloaddition by photoinitiated Cu(II) reduction, F) Photo-triggered thiol Michael-type addition by photocleavage of *o*-nitrobenzyl moieties.

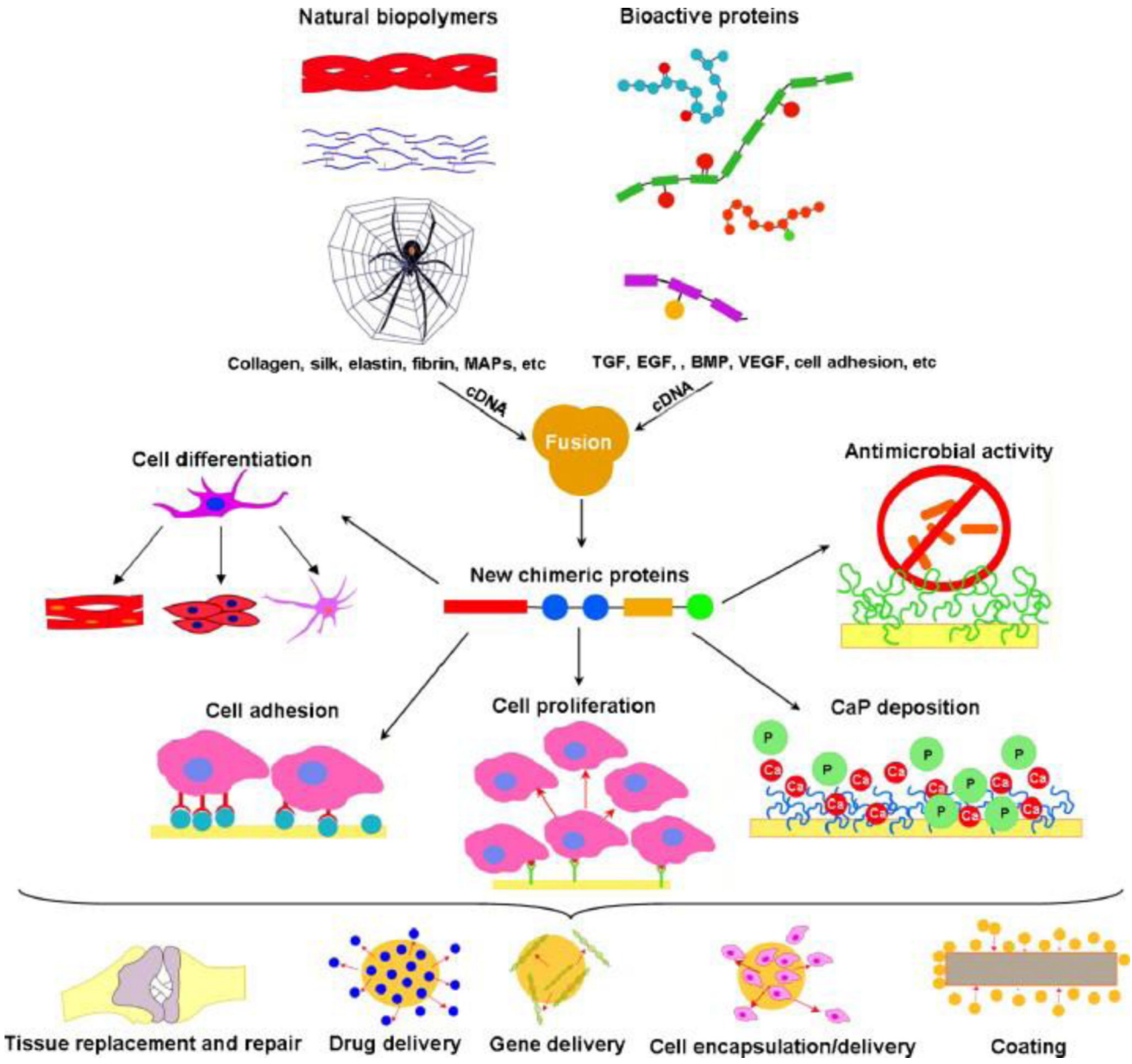

Figure 2.

Schematic of highlights of key features and potential applications of modular designed biopolymers synthesized through recombinant DNA technology. Reproduced with permission from ref 58. Copyright 2012 Elsevier.

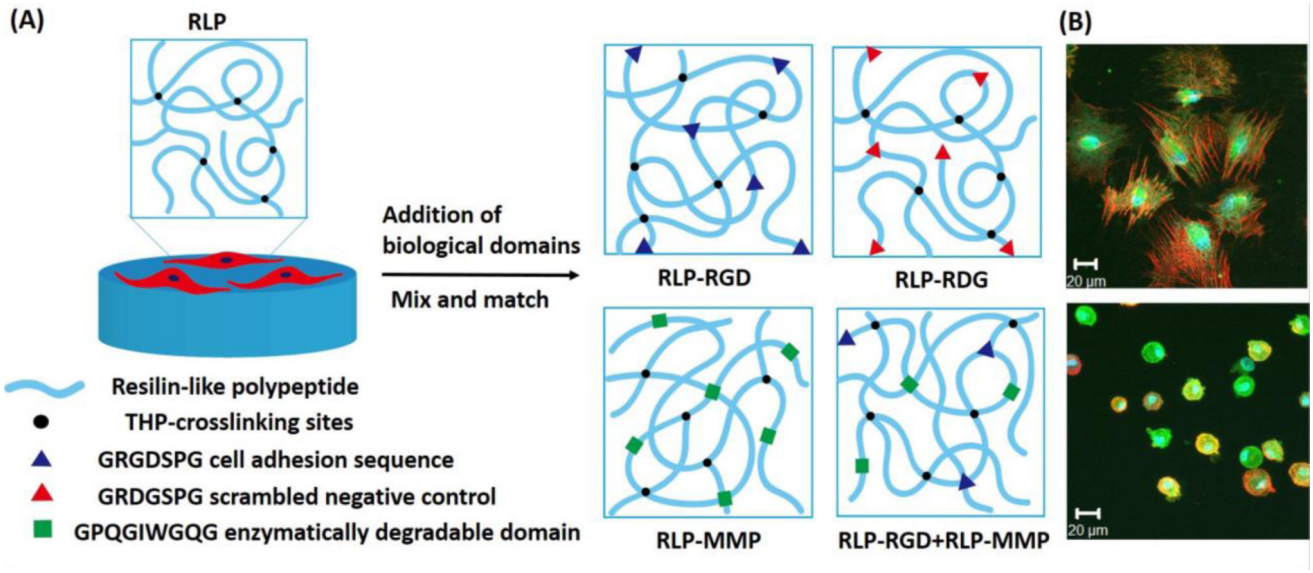

Figure 3.

Resilin-based elastomeric hydrogels with independent tunability of mechanical and biological functions for regenerative medicine application. (A) Schematic of modular RLP-based hydrogels fabricated with various material compositions. Reproduced with permission from ref 84. Copyright 2016 John Wiley & Sons, Inc. (B) representative images of hMSCs stained for visualization of nuclei, vinculin, and actin cytoskeleton, on the surfaces of RGD-containing (top) and no RGD (bottom) RLP hydrogels. Reproduced with permission from ref 88. Copyright 2013 Royal Society of Chemistry.

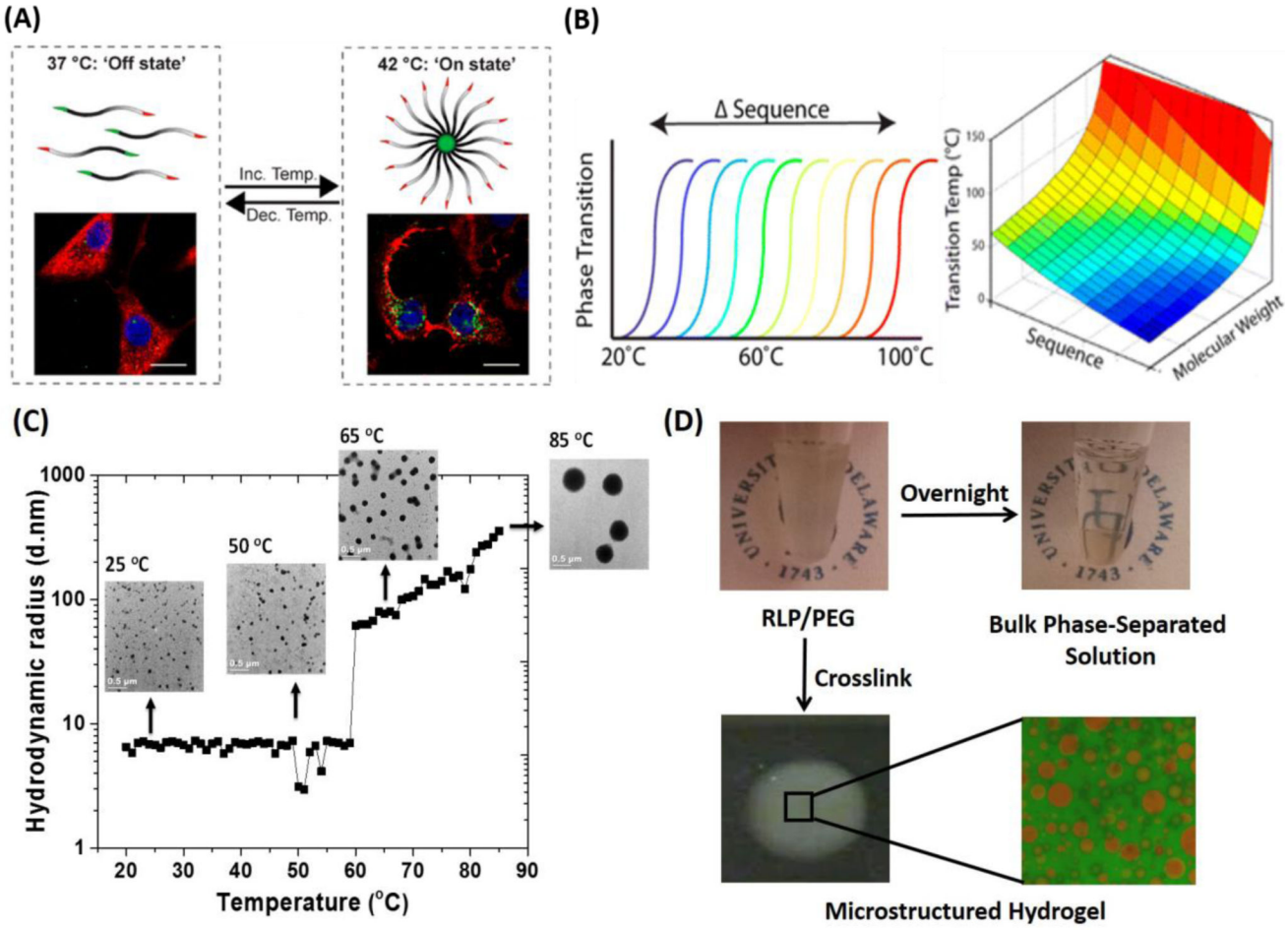

Figure 4.

Examples of multi-stimuli responsive biopolymers self-assemble to form micro- and nanostructures. (A) Digital switching of controlled cellular uptake by modulation of local arginine density is achieved by temperature-triggered micelle assembly of a genetically encoded ELP. Reproduced with permission from ref 98. Copyright 2012 American Chemical Society. (B) A 3-dimensional plot of the predicted T_t landscape for a superfamily of ELPs with various molecular weights and sequence compositions in PBS. Reproduced with permission from ref 92. Copyright 2013 American Chemical Society. (C) Temperature-triggered non-reversible LCST phase separation of a RLP with representative TEM images of RLP-based nanoparticles formed at various temperatures. Reproduced with permission from ref 109. Copyright 2015 John Wiley & Sons, Inc. (D) Solution mixtures of PEG and RLP undergo phase separation to yield two aqueous phases in PBS buffer that can be crosslinked to form micro-structured hydrogels with distinct micro domains confirmed *via* Dylight-594 labeled RLP (red) and Dylight-488 labeled PEG (green). Reproduced with permission from ref 112. Copyright 2016 American Chemical Society.

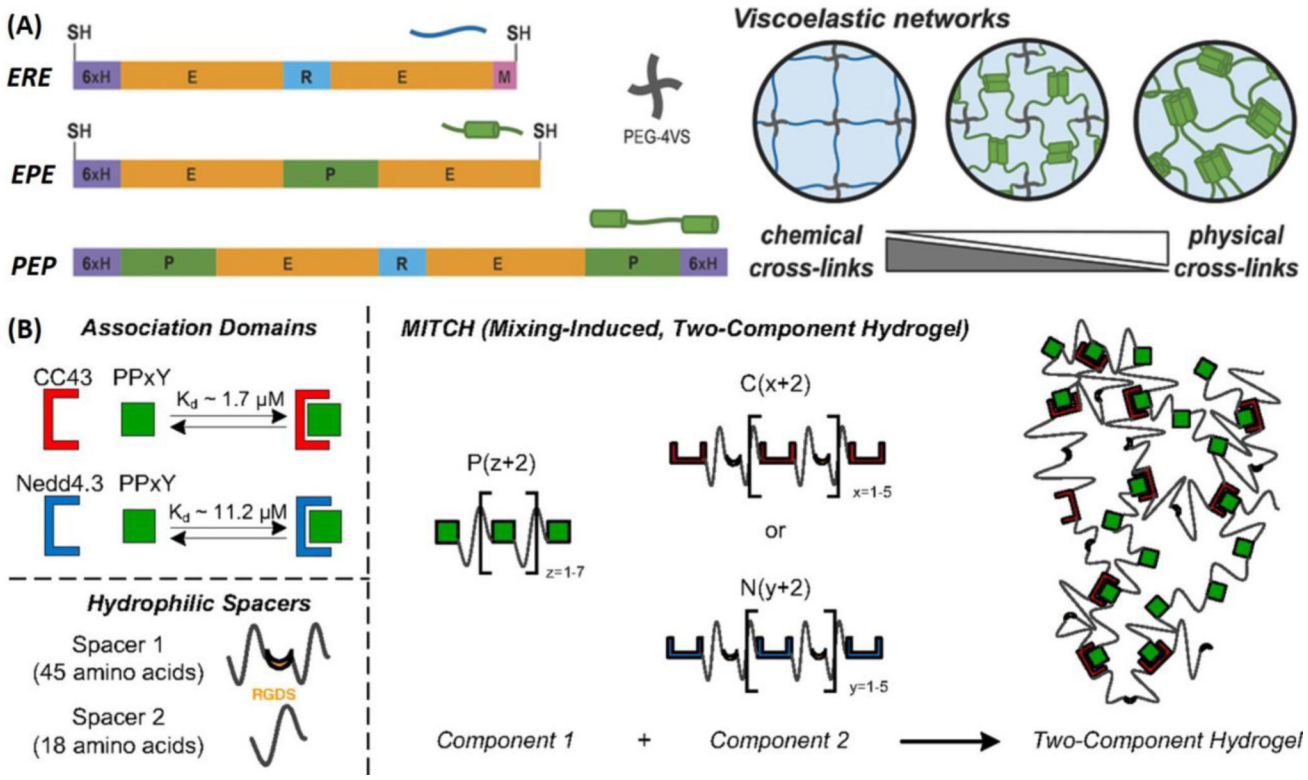

Figure 5.

Examples of modular engineered hydrogels assembled *via* reprogrammed domain association through molecular recognition. (A) Artificial proteins EPE and ERE consist of terminal cysteine residues, elastin-like end-blocks E, and either the P or R midblock domain. ERE also contains an octapeptide recognition sequence M for proteolytic cleavage. The artificial protein PEP contains two P domains near the termini that flank the elastin-RGD-elastin sequence. PEP forms physical hydrogels through association of the P end-blocks. EPE and ERE require covalent cross-linking with 4-arm PEG vinyl sulfone to form gels. ERE contains only covalent cross-links while EPE also has the potential to form physical cross-links through association of the midblock domains. Reproduced with permission from ref 117. Copyright 2016 John Wiley & Sons, Inc. (B) Schematic of the mixing-induced, two-component hydrogel (MITCH). (Top left) Two WW domains (CC43 and a Nedd4.3 variant) bind the same proline-rich peptide (PPxY). (Bottom left) Hydrophilic spacers link multiple repeats of WW domains (spacer 1) or proline peptides (spacer 2). Spacer 1 contains a cell-adhesion peptide RGDS. (Right) Three engineered protein families: C[x+2], N[y+2], and P[z+2]. Mixing component 1 with component 2 at constant physiological conditions results in hydrogel formation. Reproduced with permission from ref 119. Copyright 2009 National Academy of Sciences.

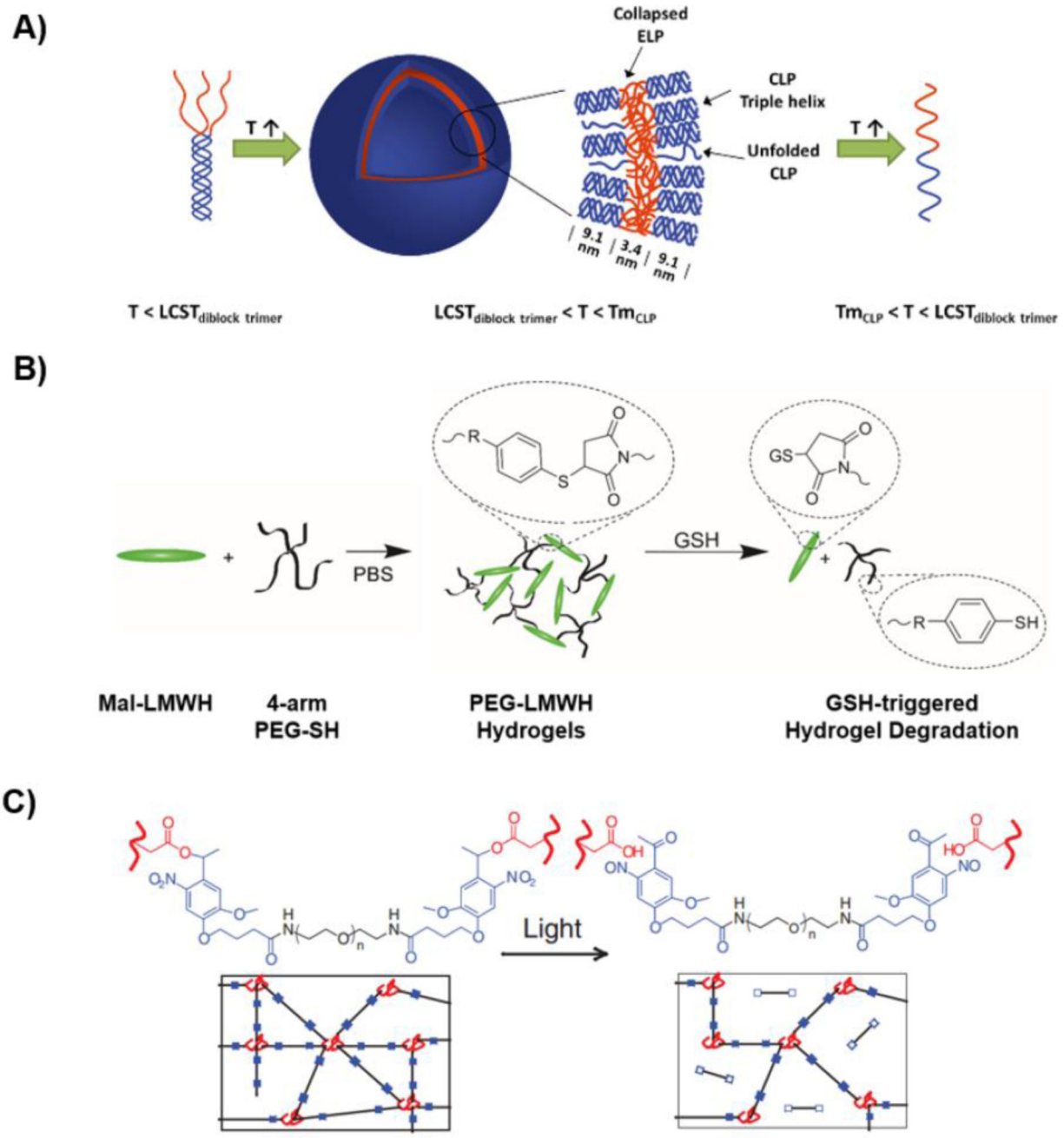

Figure 6.

Examples of stimuli-responsive biomaterials enabled by dynamic interactions/chemical linkages. A) Elastin-b-collagen-like peptide (ELP-CLP) bioconjugates with temperature-triggered assembly/disassembly behaviors. Reproduced with permission from ref 145. Copyright 2015 American Chemical Society. B) GSH-sensitive PEG-heparin hydrogels with extended degradation profiles mediated by retro Michael-type addition. Adapted with permission from ref 146. Copyright 2013, Royal Society of Chemistry. C) Photodegradable PEG hydrogels synthesized via the incorporation of a photocleavable *o*-nitrobenzyl

containing crosslinker. Reproduced with permission from ref 147. Copyright 2009 American Association for the Advancement of Science.

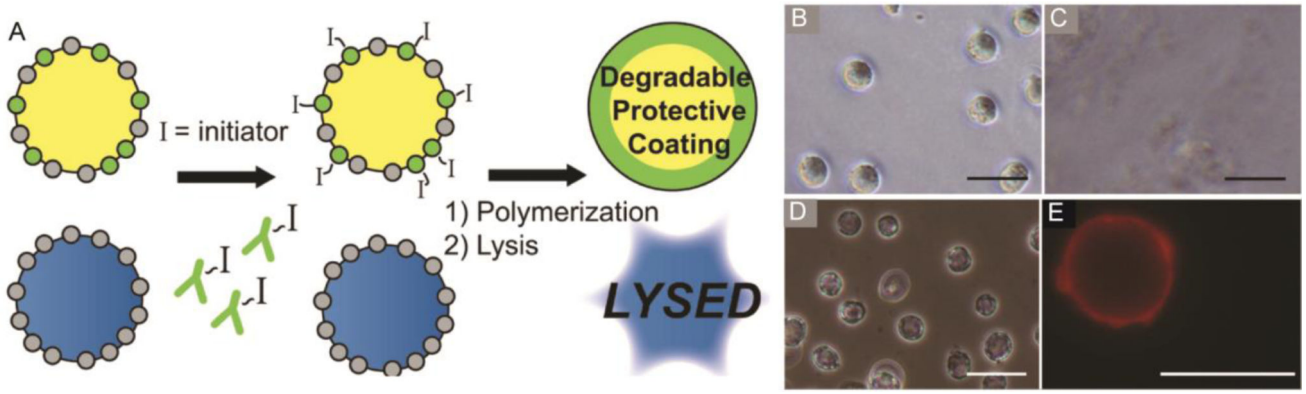

Figure 7.

Pure populations of marker-positive cells attained through polymerization. (A) Cells are immunolabeled with polymerization initiators, and protective coatings are formed only on initiator labeled cells. Unprotected cells are lysed while coated cells are viable. (B) Naive Jurkat cells. (C) Uncoated Jurkat cells are lysed in <10 s in 5% SDS. Only sparse cellular debris remains in the viscous lysate. (D) Polymer-coated Jurkats intact are after 10 min in 5% SDS. (E) Epifluorescent image of Jurkat cells coated with a red fluorescent nanoparticle-loaded polymer in pure deionized water. Scale bars are 25μm. Reproduced with permission from ref 187. Copyright 2015 American Chemical Society.

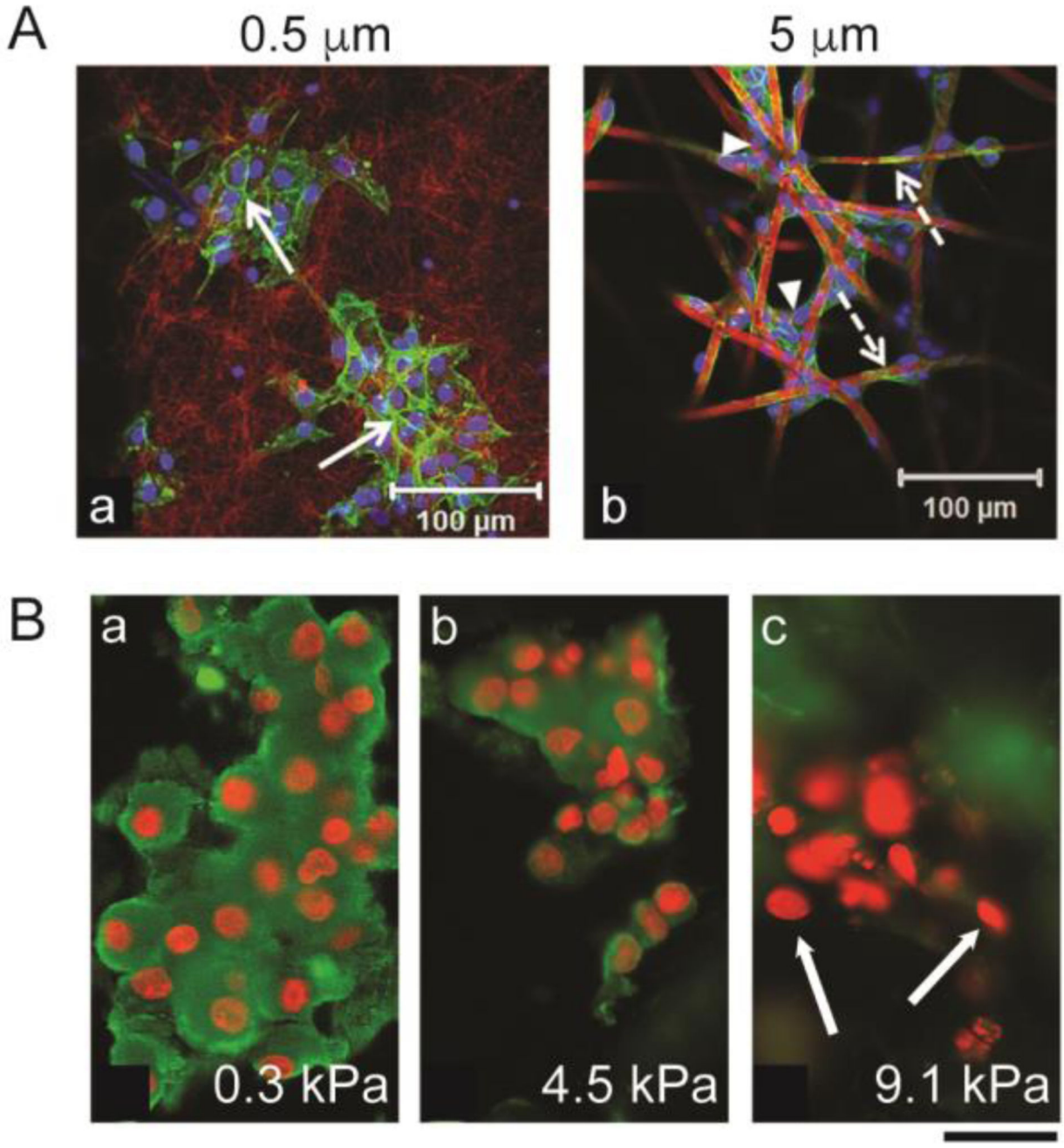

Figure 8.

Biocomplex materials direct cell response. (A) Matrix topography influences phenotype of cells. (a) MDCK (Madin-Darby Canine Kidney) cells cultured on 0.5 μm diameter PCL-RGD electrospun fibrous scaffolds grew as compact colonies with cuboidal cells, while (b) cells on 5 μm scaffolds exhibited a spindle-shaped morphology and associated more closely with individual fibers, indicative of an EMT-like phenotype. Scale bars = 100 μm. Adapted with permission from ref 208. Copyright 2016, American Chemical Society. (B). Decreasing the modulus of PEG-gelatin hydrogels induced a vascular phenotype in human cord blood stem cells. (a) In lower modulus hydrogels, cells formed large clusters and stained strongly

for von-Willebrand Factor (vWF, shown in green; nuclei are in red), an endothelial cell marker. As hydrogel modulus increased, (b) clustering became less apparent and (c) cells stained weakly or not at all for vWF. Scale bar is 50 μm. Adapted with permission from ref 217. Copyright 2015, Elsevier.