CHAPTER

19

Polymer Design and Development

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OUTLINE

1. INTRODUCTION

The therapeutic promise of stem cells, including those derived from embryos, fetuses, and adults, is unparalleled by any other approach in translational medicine.[1] Stem cells have been isolated in small numbers from a multitude of human tissues, and knowledge gained in reprogramming mature cells into induced pluripotent stem cells has opened the floodgates for their widespread clinical use. When presented with the appropriate external stimuli, stem cells will undergo lineage-specific differentiation into functional mature cells that can be used in cell-replacement therapies for many diseases including Parkinson, Type 2 diabetes, and macular degeneration. Given the tremendous potential hinging upon the directed differentiation of

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stem cells into desired cell types, intense effort worldwide has centered on the identification of specific combinations of biochemical and biophysical signals that will direct stem cells to make these functional changes. Unfortunately, obtaining such information exclusively through in vivo studies is far from trivial; the complex choreography of ever-changing signals renders it difficult to correlate observed phenotypic changes with specific cellular inputs in native tissue.

In vitro studies have proven invaluable toward expanding our understanding of cell behavior in response to well-defined extracellular cues.[2] Patient-derived stem cells can be cultured in 2D microplates and screened against massive chemical libraries to yield databases relating the complex combinations of inputs with cell fate. Though these high-throughput experiments have played a substantial role in shaping our understanding of stem cell function, there is concern that cells often respond differently to the identified cues in this format than in their natural environment. As such, there has been substantial interest in culturing and studying stem cells in formats that better mimic the chemical and physical properties of native tissue (e.g., dimensionality, matrix stiffness, water content, nutrient permeability, structural composition, cell density).[3,4] Taking lead from the native extracellular matrix (ECM) comprised of proteins and polysaccharides with repeating subunits, a variety of polymer systems have been exploited as the basis for in vitro stem cell culture. Polymeric macromolecules offer immense chemical and structural versatility based on their compositional identity, sequence, and arrangement, permitting a wide variety of polymer-based culture materials to be designed to encourage specific cell fates with vast flexibility.

In this chapter, we seek to highlight recent progress in polymer design and development for 3D stem cell culture. We will compare and contrast the advantages of polymers from natural sources, as well as those that are strictly synthetic. Special attention is given to natural polymer materials derived from polysaccharides, peptides, and proteins, as well as smart synthetic polymer systems that exhibit responsiveness to environmental stimuli (e.g., electricity, temperature, enzyme, light, heat, pH).

2. NATURAL POLYMERS FOR 3D STEM CELL CULTURE

Naturally derived polymers describe compounds generated by living organisms that have been used either in their native form or after minimal mechanical, chemical, or material processing and modification. The range of biological materials includes protein-, polysaccharide-, amino acid-, nucleic acid-, apatite-, and tissue-derived materials. As these substances exist in abundance, they are commonly inexpensive to process and manufacture. After harvesting from a natural source or a genetically modified organism, these polymers are subjected to fabrication techniques to generate scaffolds and drug delivery vehicles to manipulate cellular behavior. As both the building blocks and final structure exist within nature, naturally derived biomaterials have evolved to support cell adhesion, proliferation, migration, and differentiation by binding their natural receptors and epitopes. Furthermore, these materials exhibit little toxicity, incur minimal inflammatory responses, function and are stable in physiological conditions, are often biodegradable, and bioactive.[5] However, it is important to recognize that while these materials may be synthesized with great control in precise conformations, inaccuracies and inconsistencies in harvesting, purification, and chemical modification may infer off-target biochemical interactions, immunogenicity, and poor mechanical properties. Here, we highlight the use of both polysaccharide- and protein-based materials for stem culture.

2.1 Polysaccharide-Based Materials for Cell Culture

Naturally derived polysaccharides are simultaneously the most readily available biological polymers as well as the most complicated and least defined. In fact, polysaccharides are almost exclusively extracted from sources found in nature, as their biological synthesis is poorly understood and their chemical synthesis at the benchtop is extremely complicated. Although both polysaccharides and their monomeric constituents contain an excess of hydroxyl functional groups which can be utilized for conjugation, chemical modification can be very difficult.[6] Controlling how and where reactants will react is rarely trivial and requires significant purification after each modification.[7] Nevertheless, polysaccharides are popular choices for biomaterials as they are low cost in many forms, renewable, biodegradable, exhibit moderate biological activity, are often nontoxic and rarely immunogenic. Polysaccharide materials have been utilized as sponges, hydrogels, surface coatings, scaffolds, particles, and even targeting ligands to direct and modify cell behavior.[7,8] We will further consider cellulose, agarose, alginate, hyaluronic acid, chondroitin sulfate, and chitosan as common polysaccharide-based biopolymers that are frequently employed for 3D stem cell culture (Fig. 19.1).

2.1.1 Cellulose

Cellulose is a linear polysaccharide composed of thousands of D-glucose monosaccharides connected by β-1,4 glycosidic bonds. It is the single most abundant biopolymer on earth and is an integral and ubiquitous structural component in plant and algae cell walls. Unlike

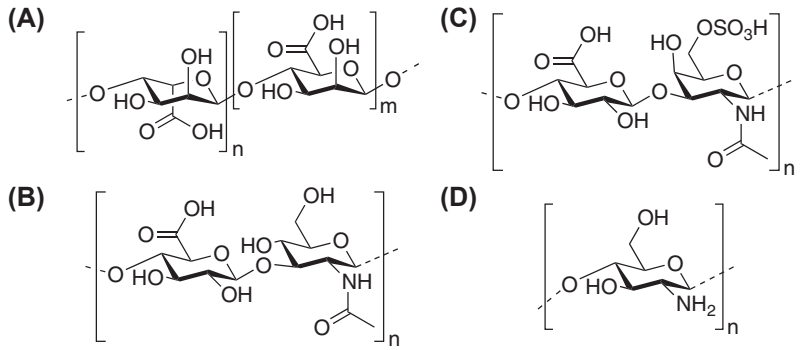

FIGURE 19.1 A variety of polysaccharides have been used as biomaterial substrates for stem cell culture. (A) Alginate, (B) hyaluronan, (C) chondroitin sulfate, and (D) chitosan.

starch, the synthesis of cellulose allows for no coiling or branches and instead exists as a single rod shaped polymer with immense tensile strength.[9] This structural uniformity infers significant crystallinity and a very high glass transition temperature of 320°C in neutral water. The glycoside hydrolase enzyme which acts to cleave β-1,4 linkages is absent in the human body and thus cellulose is considered a nondegradable material; however, modifications can be performed to cellulose to introduce biodegradability. Most notably, oxidized cellulose is a popular choice in tissue engineering as it is degradable by hydrolysis and its rate of degradation can be mediated by hydrolytic enzymes present at basal levels in the blood and secreted by macrophages.[10] Other chemical modifications include regenerated cellulose for vascular engineering,[11] acetate modification for cardiac tissue engineering,[12] and self-setting cellulose-based hydrogels for cartilage and bone tissue engineering.[13]

2.1.2 Agarose

Agarose is a linear polymer generated from repeating subunits of agarobiose, a disaccharide composed of D-galactose and 3,6-anhydro-L-galactopyranose connected by α-1,3 and β-1,4 glycosidic linkages.[7] Generally extracted from seaweed or algae, this inexpensive polymer is miscible with water and forms hydrogels quickly under very mild conditions by cooling below 4°C. Due to its highly hydrophilic nature, agarose hydrogels display high water content and therefore have been investigated for use in cartilage tissue engineering.[14] Despite early work showing promise in chrondrogenesis and minimal immunogenicity upon implantation, agarose-based hydrogels have fallen from popularity due to their bioinert nature and inability to support the growth, spreading, and proliferation of stem cells.

2.1.3 Alginate

Like agarose, alginate is also a linear unbranched polysaccharide produced by seaweed, algae, and some bacteria and thus is inexpensive and easily accessible. It consists of rigid α-1,4-linked L-guluronic acid (G blocks) and flexible β-1,4-linked D-mannuronic acid (M blocks) arranged in an irregular order. It is believed that the ratio of G to M blocks dictates local mechanical properties and modulus.[8] Though largely bioinert, alginate shows significant promise for cell encapsulation and injectable cell delivery as it forms hydrogels quickly upon cross-linking with polyvalent cationic ions (e.g., calcium, magnesium) and small molecules at physiologic conditions. Divalent cations are chelated by G-residues of close proximity generating an ionically cross-linked structure. As these adjacent polymer chains are brought closer together, significant van der Waals forces are generated, further strengthening the material.[15]

2.1.4 Hyaluronic Acid

Hyaluronic acid or hyaluronan (HA) is an anionic immunoneutral glycosaminoglycan composed of alternating units of D-*N*-acetylglucosamine and D-glucuronic acid connected by β-1,4 and β-1,3 glycosidic linkages. HA is a major component in the ECM of cartilage, the vitreous humor of the eye and epithelial-derived tissues and has been found to be a very bioactive molecule. Studies have shown that HA plays an important role in cell signaling, matrix reorganization, differentiation, and wound repair.[16] Furthermore, it is particularly appealing for applications in tissue engineering as it is readily biodegradable, broken down by endogenous hyaluronidase within days, and can be extensively modified by utilizing primary and secondary hydroxyl groups found on its glucuronic acid carboxylic acid or *N*-acetyl functional groups found on its *N*-acetyl glucosamine.[17] Derivatives of HA are subclassified into the categories monolithic (ones that cannot form new chemical bonds with biological tissue) and living (those that can participate in reactions to form bonds with cells and proteins).[18] Particular interest in the last 15 years has given rise to HA derivatives capable of forming sponges, photopolymerization, double cross-linking, and click-chemistry conjugation which have been in cartilage,

neural, bone, liver, and vascular tissue engineering, as well as tumor modeling.[17]

2.1.5 Chondroitin Sulfate

Chondroitin Sulfate (CS) is an unbranched glycosaminoglycan consisting of *N*-acetyl-D-galactosamine and D-glucuronic acid connected by β-1,3 and β-1,4 glycosidic linkages. During synthesis, the polysaccharide is sulfated in different locations along its more than 100 glycan chain and conjugated to serine residues on proteins. CS is a common constituent of cartilage and skin ECM and contributes to the viscoelastic nature of both tissues due to its high degree of hydration.[19] Hybrid polymer conjugates and polymer–polymer mixtures utilizing CS as their main component have been employed in cartilage tissue engineering, cardiac tissue engineering, dermal engineering, drug release, and for intervertebral disk regeneration.[5] On a cellular level, CS has been shown to upregulate the synthesis of hyaluronan, glucosamine, and type II collagen by both bovine and human chondrocytes while also shown to downregulate expression of enzymes responsible for cartilage ECM breakdown including MMP-1, MMP-3, MMP-13, and aggrecanase-2.[20] Recent studies have also highlighted the ability of CS to reduce inflammation by inhibiting human leukocyte chemotaxis and phagocytosis.[21] These factors have made CS a particularly important tool in the design of tissue engineering solutions to osteoarthritis.

2.1.6 Chitosan

Chitosan is a low-cost, readily available, linear polysaccharide copolymer of *N*-glucosamine and *N*-acetyl-glucosamine monomers, widely researched for applications in drug delivery, surface coating, and tissue engineering. Its precursor, chitin is second only to cellulose as the most abundant natural biopolymer.[22] Processing of chitin into chitosan is performed by deacetylating arthropod shells using concentrated sodium hydroxide, followed by purification; where chitosan is defined as those resultant copolymers with a ratio of glucosamine to *N*-acetyl glucosamine of greater than 60%.[23] Chitosan is particularly popular as it is biodegradable, biocompatible, antibacterial, and wound-healing promoting. sponges,[24] chemical and photopolymerizable hydrogels,[25] and scaffolds formed from blends of chitosan and synthetic polymers have all been utilized for stem cell culture and differentiation.[22] While other naturally derived polymers require significant chemical modification to allow for conjugation or cross-linking, chitosan's abundant free amines can participate as nucleophiles in bioconjugation reactions, making it readily modifiable. It is of note that chitosan in its native form is largely insoluble in water that is of neutral pH. Protonation of its amine groups necessitates solvent pH ranges well below physiological pH (~pH 5).[24]

2.2 Protein-Based Biomaterials for Stem Cell Culture

Given the role of ECM proteins in providing structural support and signaling cues to cells in vivo, there is extensive interest in using protein-based biomaterials for in vitro stem cell culture. Proteins represent a distinctive class of biopolymers, where genetically encoded sequences of amino acids are linked together through amide bonds to form linear, well-defined macromolecules. Unique functional groups comprising the 20 naturally occurring amino acids (whose order represents the primary structure of a protein) give rise to local conformational variation of the peptide backbone. Hydrogen bonding, hydrophobic interactions, electrostatics, and van der Waals forces govern rotation about the N-C_χ and C_α-C bonds, resulting in secondary structures including α-helixes, β-sheets, β-hairpins, and β-spirals. The ensemble of these structural motifs and the stabilizing effects of salt bridges and disulfide linkages to distant residues within a single polypeptide chain comprise a protein's tertiary structure. Multiple polypeptide chains can become further associated into complex assemblies by noncovalent bonding, giving rise to protein quaternary structure. The immense 3D structural complexity that emerges from the linear arrangement of 20 simple building blocks, coupled with their inherent biocompatibility and biofunctionality, render proteins as intriguing biopolymers for cell culture material formation.

2.2.1 Tissue-Derived Proteins for Biomaterial Formation

Owing primarily to their size and structural complexity, proteins are virtually always isolated from a living source (e.g., bacterial, plant, or mammalian), as opposed to being synthesized via nonbiologically-assisted organic methodologies. This process often involves physically isolating a tissue enriched in the protein of interest, enzymatically digesting unwanted components, lyzing cells with detergents or freeze-thaw cycles, and then purifying the final protein either by precipitation or preparative chromatographic techniques. Though these processes are somewhat involved, they have been optimized over the past several decades to afford a number of matrix proteins in high yield and purity. Here, we discuss some of the most common naturally sourced proteins used in stem cell culture, including collagen, fibrin, elastin, keratin, and silk (Fig. 19.2).

2.2.1.1 Collagens and Gelatin

Collagens represent a family of fibrous proteins found in nearly every mammalian tissue that provides

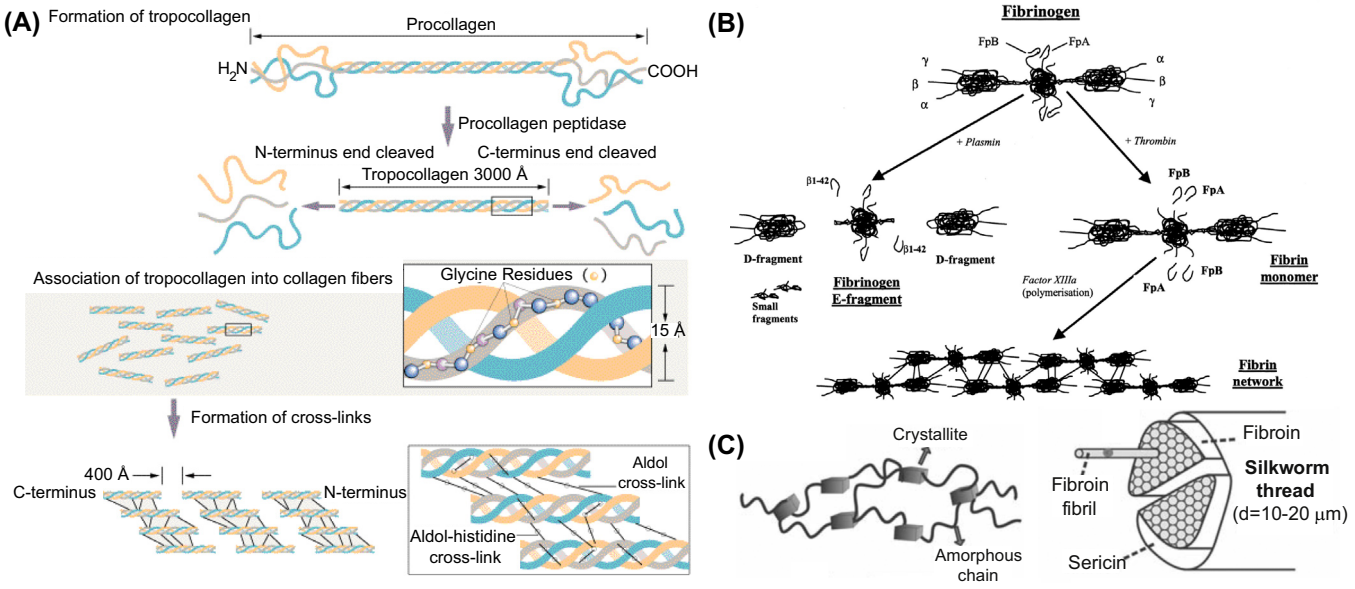

FIGURE 19.2 The natural assembly mechanisms of tissue-derived proteins have been used for biomaterial formation. (A) Collagen fibers are formed from the self-assembly of tropocollagen. (B) An enzymatic cleavage of fibrinogen exposes polymerization sites that can form electrostatic bonds between adjacent molecules. Factor XIIIa further introduces chemical cross-links to form the fibrin network. (C) Fibrils that containing both crystalline and amorphous segments organize into a hierarchical structure making up silk fibers. *Modified from Klug WS, Cummings MR, Shotwell M, Spencer C.* Concepts of genetics. *5th ed. Upper Saddle River (NJ): Prentice Hall; 1997; Bootle-Wilbraham CA, Tazzyman S, Marshall JM, Lewis CE. Fibrinogen E-fragment inhibits the migration and tubule formation of human dermal microvascular endothelial cells in vitro.* Cancer Res *2000;**60**(17): 4719–4724; Du N, Yang Z, Liu XY, Li Y, Xu HY. Structural origin of the strain-hardening of spider silk.* Adv Funct M*ater 2011;**21**(4):772–778.*

mechanical support and structural integrity to the ECM. Though collagen production and secretion by resident fibroblasts is highly expressed in load-bearing tissues such as tendon and bone, these proteins are also abundant in skin, cartilage, and a number of internal organs. Collagens are found not only at many locations within an organism, but also in highly conserved sequences and structures that span virtually all vertebrates. Such sequence homology has enabled collagen to be extracted from a variety of animal sources (e.g., cows, pigs, rats) to be used in humans with only relatively mild immune response.

At least 28 isoforms of collagen have been identified, composed of >40 distinct polypeptide chains.[26] Over 90% of the collagen in the human body is of Type I, which is found in skin, tendon, vascular ligature, and bone. Type II is derived from cartilage; III from reticular fibers in skin and blood vessels; IV from basement membrane; and V from cell surfaces as well as hair. Common to all types, collagen molecules self-assemble from three α chains as a result of their molecular structure. Each polyproline peptide α chain contains thousands of repeats of the -X-Y-Gly- sequence, where X and Y can be any amino acids but are most commonly proline and non-proteinogenic hydroxyproline (Hyp) residues that drive left-handed chain helicity.[27] The glycine residue is critical in enabling tight packing and assembly of three α chains into a single tropocollagen molecule, a right-handed triple helix-containing structure stabilized by intermolecular hydrogen bonding. Repeating sequences within collagen α chains are flanked by ~20 amino acid-long telopeptides that deviate from this repeating structure and therefore do not participate in helix formation. All told, these α chains exhibit a fairly narrow molecular weight distribution centered around 100 kDa. The tropocollagen rods can self-assemble with a quarter-staggered structure into fibrils. These fibril structures exhibit uniquely high strength, in part due to intermolecular cross-links that form either enzymatically or nonenzymatically between adjacent telopeptide regions in the extracellular space. Fibrils further self-assemble into inelastic load-bearing collagen fibers with low extensibility (~15%) that provide structural support to many mammalian tissues.[28]

Given the hierarchical structure comprising collagen fibers, a variety of degradative processes (e.g., enzymatic, acid extraction, salt treatment) must be employed to isolate collagen tropohelices or stable α chains from collagenous tissue. Pepsin is often employed to enzymatically cleave the cross-linked telopeptide regions from the collagen fibers, ultimately producing soluble triple helices known as atelocollagen. Additionally, treatments with either acid or salt can be used to disrupt tropocollagen hydrogen bonding, again yielding soluble chains through structural dissolution. Denatured α

chains can be stabilized as gelatin, a popular material for stem cell culture that forms a thermoreversible gel upon cooling. When acid-denatured collagens are returned to physiological pH and temperature, self-assembly of collagen fibers can occur once more. This property is often exploited to create collagen-based gels for 2D and 3D cell culture.[26,29] Such materials are commercially available as PureCol, SureCoat, and VitroCol from Advanced BioMatrix.

Unlike many of the other polymeric materials that we highlight here, collagen contains a variety of biological motifs capable of directly promoting cell attachment, proliferation, and even differentiation of cultured cells. Additionally, protein-sequestering motifs are present that bind fibronectin, decorin, and laminin, providing a substrate that promotes cell adhesion and proliferation through integrin-mediated signaling.[30]

2.2.1.2 Fibrin

Fibrin represents a naturally occurring hemostatic biopolymer matrix of the blood plasma monomer fibrinogen that is useful for 3D stem cell culture. Unlike collagen-based networks that assemble slowly in an orderly fashion, fibrin-based networks form quickly through a modified polycondensation reaction of fibrinogen. Fibrinogen is a 45-nm long glycoprotein comprised of two sets of three polypeptide chains (Aα, Bβ, and γ) held together by 29 disulfide bonds.[31] The six chains are held together in a central region by α-helical coiled-coils, and contain two pairs of fibrinopeptides A and B.[32] Upon tissue trauma and blood clotting initiation, the protease thrombin is activated and cleaves fibrinopeptides A and B from fibrinogen to expose "knob" polymerization sites complementary to other portions of the fibrin monomer.[33] The conformational change of the glycoprotein monomer enables homopolymerization in a half-staggered fashion. Upon oligomerization, two-stranded micron-long semiflexible protofibrils are formed. These protofibrils subsequently aggregate into fibers. During fiber formation, branching can occur, enabling 3D gels to form (as opposed to linear structures). These 3D networks are further stabilized by plasma transglutaminase factor XIIIa, which catalyzes ligation of glutamine and lysine side chains of neighboring fibers, rendering the networks resistant to proteolytic degradation.[34]

Given the abundance and ease of purification of both thrombin and fibrinogen from blood plasma, fibrin gels have gained much use as cell culture matrices. Network structure, including degree of branching and fiber thickness, is easily turned by the polymerization conditions. Through simple changes in temperature, species concentration, pH, and ionic composition, matrices with a variety of different stiffness and porosity can be readily generated.[35] Moreover, fibrin networks provide a plethora of natural cell signaling cues. Binding sites for cell-surface integrins, growth factors, and various ECM species (e.g., fibronectin, thrombospondin) are present on fibrin. The networks are biodegradable and can be enzymatically broken down by the fibrinolytic system, as well as other nonspecific proteases. These biopolymer systems continue to hold great promise for applications in regenerative medicine and stem cell culture.

2.2.1.3 Elastin

Elastin represents another ECM protein that has gained popularity as a tissue culture matrix material. This elastomeric structural protein is important for providing elasticity and resilience to tissues and organs that undergo reversible stretching, and is abundant in blood vessels, lung, skin, and ligaments.[36] It is synthesized by several cell types (e.g., smooth muscle, endothelial, fibroblasts, keratinocytes) as a 72 kDa soluble precursor known as tropoelastin.[37] Similar to collagen, elastin contains roughly 30% glycine residues and a substantial fraction of proline. Unlike collagen, where the remaining amino acids are largely hydrophilic, elastin is characterized by alternating hydrophobic and hydrophilic domains. Hydrophilic regions, which are important in monomer cross-linking, are enriched in lysine or alanine and often contain the repeating sequence -Ala-Ala-Ala-Lys-Ala-Ala-Lys-Ala-Ala-. The hydrophobic domains are dominated by glycine, proline, and valine (e.g., repeats of -Gly-Val-Gly-Val-Pro- or -Gly-Val-Gly-Val-Ala-Pro-) and are important in driving tropoelastin coacervation.[38] During this process, tropoelastin monomers concentrate and align to generate tetrafunctional cross-links between adjacent lysine residues, resulting in elastin self-assembly and insoluble fiber formation. The resulting structures exhibit significant extensibility and high resilience upon stretching.

In addition to its unique elastomeric features, elastin can also promote a variety of cell functions including attachment, proliferation, migration, and differentiation.[39] It is also a known chemoattractant for endothelial cells and smooth muscle cells.[40] Though less has been done to date with these biopolymer systems than collagen or fibrin, elastin-based networks are gaining significant popularity with a number of researchers due to their unique mechanical properties.

2.2.1.4 Silk

Silks represent naturally occurring fibrous protein polymer materials created by several insects and spiders for a variety of functions (e.g., web construction, capture of prey, reproduction).[41] The filament core of silk is comprised of a protein known as fibroin, a naturally occurring block copolymer of alternating hydrophobic and hydrophilic regions.[42] The hydrophobic segments are highly repetitive, consisting primarily of glycine

and alanine residues. Hydrophilic segments have more complex sequences and contain charged amino acids including serine and aspartic acid. The hydrophobic portions self-assembled into stable crystalline regions primarily comprised of β-sheets through hydrophobic interactions and hydrogen bonding. Hydrophilic regions limit some of these interactions, rendering these tough fibroins moderately elastic. Coating this core and enabling fibroin fibers to stick to one another is a glue-like mixture of sericin proteins. This unique structure gives rise to silk's unique physical properties: high strength-to-weight ratio, toughness, and elasticity.[43]

Silk is most often harvested from the Chinese silkworm *Bombyx mori*. This material can be woven or electrospun into fibrous net membranes, or further processed to generate film, hydrogels, and sponges for stem cell culture. The resulting scaffolds are relatively biocompatible and biodegradable, while providing structural support to encapsulated cells.[44]

2.2.1.5 Complex Mixtures of Natural Proteins

Though we have thus far highlighted single-protein-based materials for 3D stem cell culture, several popular protein mixtures also are well equipped for this purpose. Matrigel is perhaps the best known and represents a heterogeneous mixture of gelatinous proteins that is secreted and isolated from Engelbreth-Holm-Swarm (EHS) mouse sarcoma cells.[45] Laminin, collagen, and entactin are the primary protein species present, though proteoglycans and growth factors (e.g., transforming growth factor β, epithelial growth factor) are also abundant.[46]

Decellularized ECM (dECM) has become a popular option as a heterogenerous culture medium.[47] Here, native tissue is flushed with surfactant to remove inhabiting cells and their DNA.[48,49] The resulting materials can either be directly repopulated with stem cells, or processed into a complex mixture used as the basis for hydrogel formation or 3D printing applications. Though cell response to heterogeneous protein mixtures like Matrigel and dECM is generally quite favorable, it should be noted that these materials vary significantly from lot to lot and may not be appropriate for experiments where knowledge of precise material composition is required.

2.2.2 *Recombinant Proteins for Biomaterial Formation*

Though cells often thrive in biomaterials comprised of tissue-derived proteins, batch-to-batch variability in their origin and isolation protocol makes it difficult to standardize resulting materials. Moreover, the physical and chemical properties of such materials are difficult to engineer, often to the point of irreproducibility. To address these concerns, researchers have exploited recombinant techniques where single protein components can be expressed, purified, and employed as the basis for stem cell niche matrices.[50–53] Recombinantly expressed proteins represent perfect biopolymers, as they can be readily created with a defined length and sequence from user-specified DNA templates. Moreover, the purification from these simplified systems yields exceptionally pure protein with decreased contamination risks compared with proteins derived from animal tissue. Two primary classes of recombinant proteins that have been used for biomaterial development will be considered here: self-assembling artificial proteins and biomimetic proteins.

2.2.2.1 Self-Assembling Artificial Proteins

Early research focused on the development of self-assembling artificial proteins through coil–coil interactions. Telechelic triblock proteins containing a random coil polyelectrolyte chain flanked with terminal leucine zipper domains have been demonstrated to exhibit reversible gelation.[54,55] These shear-thinning materials can be injected and display self-healing properties under physiological conditions. At high temperatures or nonneutral pHs, leucine zippers unfold, preventing coil–coil association and material gelation. Additionally, diblock protein amphiphiles containing hydrophobic and charged segments self–associate via charge–charge interactions to form shear-thinning hydrogel materials.[56] Others have examined the use of two-component protein-based hydrogels that self-associate upon mixture through molecular recognition events.[57,58] In the first demonstration of this approach, physical cross-linking occurs between a tryptophan-rich domain and a proline-rich domain and results in stable, noncovalent strand association. By varying protein stoichiometry and sequence, final properties of the material were readily tunable. Unfortunately, each of these systems have suffered from weak overall mechanical properties and undesired gel erosion in open solution. Chemical cross-linking can be employed to yield more stable materials. Covalently-crosslinked two-component protein materials have been created based on the spontaneous isopeptide linkage between lysine and aspartic acid residues of genetically encoded reactive partners SpyTag and SpyCatcher.[59]

2.2.2.2 Biomimetic Recombinant Proteins

Biomimetic approaches have also gained popularity where repeating sequence motifs of tissue-derived structural proteins are engineered into recombinant platforms. By defining the primary sequence of the expressed proteins to match those found in nature, bioactive hydrogel networks can be readily formed via the same noncovalent interactions but in proteins where the exact material composition is explicitly known. Initial efforts in this regard have focused on mimicking

self-assembling protein systems that have already proven useful for 3D cell culture. Perhaps the most popular systems to date have been based on the expression and assembly of elastin-like polypeptides (ELPs).[60] Here, biosynthetic proteins are comprised of repeating pentapeptide sequences of -Val-Pro-Gly-Xaa-Gly-, where Xaa is any guest residue other than proline. At low temperatures, ELPs exhibit a random coil confirmation and are fully soluble in aqueous solutions. At elevated temperatures, ELPs undergo conformational transformation to form β-spirals, which further leads to protein aggregation and 3D gel formation through tertiary interactions. This transition temperature (known as the lower critical solution temperature, LCST) can be tuned through alterations in sequence repeats, protein concentration, and guest residue composition.[61,62] Temperature cycling about the LCST offers a simple, scalable, low-cost way to purify ELP proteins, making it straightforward to generate large amount of pure protein for cell encapsulation.[63,64] Moreover, additional bioactive regimes can be included into multiblock ELP-containing proteins.[65] Recombinant approaches have also been exploited to create biomaterial systems based on resilin, a related elastomeric protein.[66]

Silk-like recombinant proteins have been created and utilized for stem cell culture. The majority of these approaches utilize repeating sequences derived from dragline spider silk (-Gly-Ala-Gla-Ala-Ser- or -Gly-Pro-Gly-Xaa-Xaa-). Unlike that from the Chinese silkworm, spider silk is difficult to farm and must be produced recombinantly to achieve the large quantities necessary for biomaterial development.[67] The assembled proteins display high strength and toughness while still remaining biodegradable, rendering them excellent choices for cell culture.[68]

2.2.3 *Self-Assembled Oligopeptides for Biomaterial Formation*

Though naturally sourced and recombinant protein-based biomaterials represent effective constructs for stem cell culture, there is also interest in preparing hydrogels from synthetic oligopeptides. These simpler systems can be considered advantageous in their ease of synthesis and material formation, reproducibility, and limited risk of introducing biological contamination into the cell culture. Moreover, solid-phase synthetic techniques can be used to create peptides readily that contain nonnatural functionality, effectively expanding the toolset by which biomaterials can be assembled. Typically, well-designed oligopeptides assemble into parallel or perpendicular nanofibers of 1−10 nm diameters, which further entangle to form physically cross-linked materials.[69] Though current synthetic methodologies limit oligopeptide length to ~50 amino acids, these simple molecules have proven useful for tissue engineering applications. Here, we will highlight systems based on β-sheet structures, collagen mimicking sequences, and peptide amphiphiles (Fig. 19.3).

2.2.3.1 Self-Assembled Oligopeptides Based on β-Sheet Structures

β-sheet assembly represents one of the most common methods to produce oligopeptide-based materials serving as model stem cell niches. β-strands formation occurs when hydrophilic and hydrophobic peptide side chains are positioned on opposite sides of a peptide backbone. Hydrogen bonding between individual strands promotes association into intermolecular β-sheets that contain both a hydrophilic and a hydrophobic face. In the presence of millimolar concentrations of salt, these sheets will further assemble into filaments, which entangle to form physically cross-linked networks of supramolecular fibers. Common sequences exhibiting this behavior include (Ala-Glu-Ala-Glu-Lys-Ala-Lys)$_2$ and (Phe-Glu-Phe-Glu-Phe-Lys-Phe-Lys)$_2$.[70] These peptide-based materials promote 2D cell attachment and 3D cell spreading and are commercially available as PuraMatrix from Corning.

Hydrogel culture materials have been developed that can undergo externally triggered gelation through β-sheet formation. Most commonly, intramolecular folding of a random coil peptide chain into a β-hairpin enables intermolecular β-sheet self-assembly, fibril growth, and hydrogel formation. By controlling peptide folding through changes in pH, salt, temperature, and light, on-demand physical gelation of these peptide-based cell culture platforms has been obtained.[69] Control over gelation kinetics and material stiffness, and enabling homogeneous encapsulation of living stem cells is readily achieved through changes in these externally defined stimuli.[71]

Though the examples discussed above have involved gelation of oligopeptides of 10−20 amino acid residues, β-sheet rich hydrogels have also been demonstrated through the self-assembly of shorter dipeptide and tripeptide systems.[72] In the most extensively characterized systems, dipeptides such as Phe-Phe are functionalized with Fmoc (9-Fluorenylmethoxycarbonyl), a bulky hydrophobic moiety containing conjugated aromatic groups. Neighboring antiparallel β-sheets are held together through Fmoc π-π stacking to yield nanotubes that further entangle to form physical networks. The self-assembly of short peptides are particularly attractive from a cell culture perspective as material precursors can be readily produced inexpensively on a large scale with high purity.

2.2.3.2 Self-Assembled Oligopeptides Based on Collagen Mimicking Sequences

As collagen represents one of the most utilized natural polymers for stem cell culture, there is interest in developing oligopeptide-based materials that provide

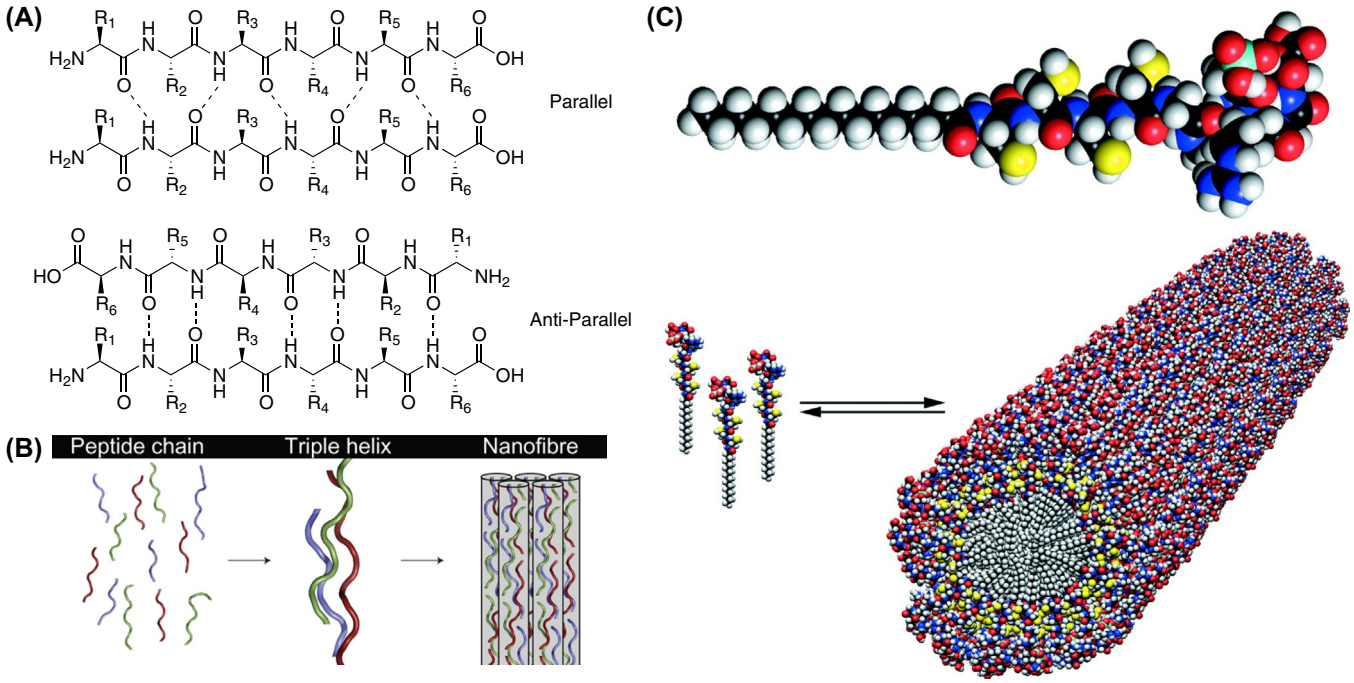

FIGURE 19.3 Biomimetic self-assembly of oligopeptides has been used for biomaterial formation. (A) Peptide chains stabilized as parallel and antiparallel β-sheets by hydrogen bonding. (B) Collagen mimicking sequences can self-assemble into staggered triple helical structure that can form nanofibers through helix elongation and lateral packing. (C) Peptide amphiphiles can self-assemble into peptide-presenting cylindrical micelles. *Modified from O'Leary LE, Fallas JA, Bakota EL, Kang MK, Hartgerink JD. Multi-hierarchical self-assembly of a collagen mimetic peptide from triple helix to nanofibre and hydrogel.* Nat Chem *2011;**3**(10):821–828; Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers.* Science *2001;**294**(5547):1684–1688.*

similar hierarchical structure and biological properties but with fully defined components. Early research sought to create materials from repeating tripeptides of Gly-Pro-Pro and Gly-Pro-Hyp, matching common sequence motifs in native collagen. Though self-association and helical formation occurs readily, assembly into fibers and hydrogel materials has not yet been observed for these simple sequences.[73] Next generation systems that further undergo hierarchical assembly into hydrogels have been synthesized using a zwitterionic sequence (Pro-Lys-Gly)$_4$(Pro-Hyp-Gly)$_4$(Asp-Hyp-Gly)$_4$.[74] Here, salt-bridged hydrogen bonds between Lys and Asp stabilize triple helix formation in a sticky-ended assembly into nanofiber-based hydrogels containing the characteristic collagen triple helical packing. Use of these systems for 3D cell culture continues to be of interest.

2.2.3.3 Self-Assembled Oligopeptides Based on Peptide Amphiphiles

Biomaterial formation of many protein-based systems relies on the amphiphilic properties of its precursors. Though hydrophilic and hydrophobic residues can be included on peptide chains, organic species can introduce lipophilicity.[75] Peptide amphiphiles (PAs) represent a class of molecules that exhibit the structural features of amphiphilic surfactants with the biofunctionality of peptides. Most commonly, PA molecules contain a hydrophobic alkyl tail, a short peptide that can form hydrogen bonds with other peptide segments, a hydrophilic block for solubility, and a bioactive headgroup.[69] In appropriate aqueous solutions, the alkyl tails can undergo hydrophobic collapse to spontaneously form high aspect ratio nanofibers with surface-displayed peptides. Nanofibers can be further cross-linked into hydrogel materials through a variety of chemistries (e.g., disulfide formation, UV cross-linking).[76] User-selected biofunctionality can be included within the system through the sequence selection of the bioactive headgroup. Peptide epitopes for cell adhesion, growth factor sequestration, and heparin-binding have all been included, making materials based on PAs particularly versatile for 3D stem cell culture.

3. SYNTHETIC POLYMERS FOR 3D STEM CELL CULTURE

Synthetic biomaterials are unique in their capacity to be very well defined in molecular weight, mechanical properties, reactivity, and degradation kinetics. By completely synthesizing and engineering a polymer,

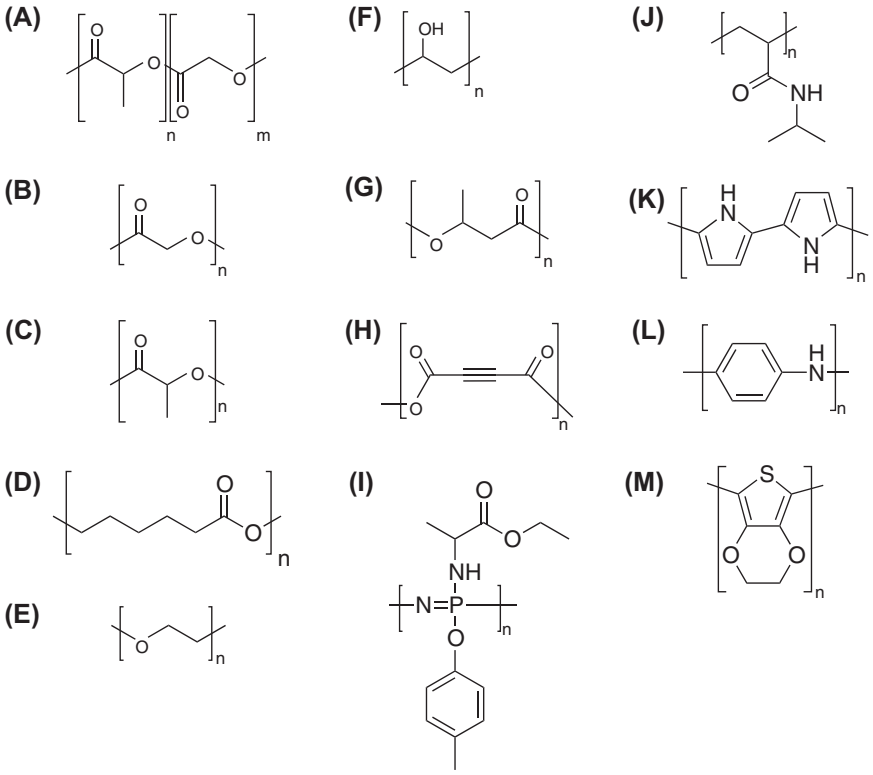

FIGURE 19.4 Several synthetic polymers have been used in the creation of biomaterial systems for cell culture. (A) Poly(lactic-co-glycolic acid), (B) polyglycolic acid, (C) polylactic acid, (D) polycaprolactone, (E) poly(ethylene glycol), (F) poly(vinyl alcohol), (G) poly(3-hydroxybutyrate), (H) polyanhydride of acetylenedicarboxycylic acid, (I) poly[(ethyl alanato)(1)(p-methyl phenoxy)(1)] phosphazene, (J) poly(*N*-isopropylacrylamide), (K) polypyrrole, (L) polyaniline, and (M) poly(3,4-ethylenedioxythiophene).

one can fine-tune material properties in a way that is reproducible and well understood.[77] Such materials can be engineered to mimic features of naturally occurring polymers and be integrative with native biology or be designed to respond to nonnatural external stimulation and polymerize through a bio-orthogonal mechanism.[78] Cost, availability, and manufacture are dependent on the complexity of the synthetic monomer, polymerization mechanism, and length of synthesis.[79] While many of the mechanical properties and chemical reactivity of a synthetic polymer can be determined a priori, these polymers are subject to unpredictable immunologic host responses. Should such a material be biodegradable, biocompatibility and clearance must be determined for both polymer and monomeric degradation products.[80] Our discussion will highlight the use of aliphatic polyesters, poly(ethylene glycol), poly(vinyl alcohol), elastomers, polyanhydrides, and polyphosphazenes as common synthetic polymer systems that have shown promise in mimicking the stem cell niche (Fig. 19.4).

3.1 Aliphatic Polyesters

The class of aliphatic polyesters are one of the most widely used types of biodegradable polymers. As the name implies, these polymers are composed of straight chained or branched carbon-based monomers, connected with hydrolyzable ester linkages. Of particular interest in tissue engineering are those composed of glycolic, lactic, and hydroxycaproic acid derivatives.[19] These monomers are either found in nature as byproducts of metabolism or have been proven to be safely cleared from the body after degradation. Aliphatic polyester polymers are synthesized through a two-step process in which acids are converted into lactones and then participate in ring-opening polymerizations.[19] Degradation occurs via hydrolysis until polymer chains are on the order of 5 kDa at which point they become water-soluble. As aliphatic polyesters are among the most common biodegradable polymers utilized in tissue engineering, their use, degradation, and the immunologic response they elicit is well understood.[81] For these reasons, they are commonly used in implantable devices and are readily FDA approved.[82] Some popular examples of aliphatic polyesters that have been used as stem cell culture materials are given below.

3.1.1 Poly(lactic-co-glycolic acid)

Poly(lactic-co-glycolic acid) (PLGA) has historically been the biodegradable material of choice for tissue grafts, tissue engineering scaffolds, nanoparticles, sutures, and resorbable prosthetic devices.[83] PLGA is

a copolymer constructed from the naturally occurring byproducts of metabolism: glycolic and lactic acid. As these monomers are regularly generated and eliminated from the human body, the material is well tolerated, exhibiting minimal systemic toxicity and a localized immunological response.[81] PLGA is especially appealing as one can alter the degradation rate of the material by altering the ratio of glycolic acid to lactic acid during polymer synthesis, where higher glycolic acid contents yield faster degradation.[84]

3.1.2 Polyglycolide

Polyglycolide (PGA) is a hydrolytically degradable, thermoplastic, highly crystalline polymer (roughly 40−50% in crystallinity) most widely noted for its use in biodegradable surgical sutures. PGA fibers due to their crystallinity can exhibit a Young's modulus of upward of 7 GPa. Molecular weight has dramatic effects on the solubility of the polymer chain, as it has been noted that high molecular weight PGA is largely insoluble in either aqueous or organic solvents, while lower-molecular weight PGA is readily soluble in these same solvents. Sutures fabricated from PGA are designed to be resorbed anywhere from 60 to 90 days.

3.1.3 Polylactic acid

Similar to PGA, polylactic acid (PLA) is composed of a naturally occurring metabolic byproduct, lactic acid. While also hydrolytically degradable and thermoplastic and exhibiting a Young's modulus between 5 and 9 GPa, PLA exhibits a significantly slower degradation rate as compared with PGA. Being stiff and degrading over 6 months to 2 years while exhibiting good biocompatibility, PLA has been used most often as screws, plates, rods, and bone tissue engineering devices, as well as filament for 3D printing.[85,86]

3.1.4 Polycaprolactone

Polycaprolactone (PCL) is a widely used hydrolytically degradable polymer synthesized through a ring-opening polymerization of ε-caprolactone. As the polymer is both hydrophobic and semicrystalline, complete degradation of the polymer can take as long as 3−4 years, making PCL a popular choice in long-term implants, bone tissue engineering, and slow releasing drug delivery applications.[87] The greatest attribute of PCL, however, is its versatility in processing and chemical properties. Its low melting temperature allows for great malleability, 3D printing capacity, heat molding, and shape memory.[88] Even more importantly, its excellent solubility in common organic solvents and ability to readily form polymer−polymer blends allows PCL to be mixed with faster degrading polymers to tune both mechanical properties and degradation time.[89] The solubility properties of PCL have been most notably utilized in electrospinning to generate sheets, tubes, and sponges with nanotopography consisting of parallel- or randomly oriented fibers.[90] Although cell adhesion is poor on PCL surfaces, collagen coating of PCL is often used to promote cell attachment.

3.2 Poly(ethylene glycol)

Poly(ethylene glycol) (PEG) is among the most well-understood and utilized synthetic polymers, and has been FDA approved as a material for numerous implantable devices, surface coatings, and polymer drug conjugates. PEG is hydrophilic, unlike polybutylene oxide or polymethylene oxide (polymers containing one more or one less methylene group than PEG, respectively).[91] This unique property is believed to be a product of the specific length of the PEG polymer that allows for thermodynamically favorable interactions with individual water molecules. As a result, PEG chains create a "hydrated" layer,[91] masking the underlying bulk material and protecting it from proteins, including blood coagulants, cell surface binding motifs, and antibodies.[92] PEG is furthermore extremely versatile as it can readily be chemically modified with a myriad of functional groups, used to create complex dendrimers and polymers with well-defined molecular weights, and altered to promote cell attachment or protein conjugation. In tissue engineering and stem cell culture, PEG hydrogels have emerged as one of the most successful synthetic polymer systems, paving the way to study and understand effects of the extracellular niche on stem cell fate. Early research concentrated on the development of PEG diacrylate monomers cross-linked together through thermo- or photopolymerization.[93] While nondegradable in its most basic form, PEG polymers can be cross-linked using degradable polymer or peptide chain cross-linkers to become biodegradable or can be conjugated with pendant conjugates to become more bioactive. Using these and many other techniques, PEG hydrogels have been optimized to culture stem cells from functional cartilage, bone, neural tissue, and pancreatic islets.[94]

3.3 Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is a simple hydrophilic biodegradable polymer containing a single hydroxyl group per monomer polymerized from vinyl acetate followed by hydrolysis. It is important to note that the hydrolysis of the precursor poly(vinyl acetate) depends on the molecular weight of the polymer and thus PVA is most often a mixture of poly(vinyl alcohol) and poly(vinyl acetate) [where a high-grade PVA may exist as 99% poly(vinyl alcohol)]. As PVA is most often synthesized using radical polymerization, the resulting polydispersity index can be quite high (as much as 5) but can

be purified using standard chromatography techniques.[95] PVA is most commonly utilized as a hydrogel or as a sponge and can be chemically cross-linked using small-molecule bifunctional cross-linking agents like glutaraldehyde or through gamma irradiation or can undergo repeating freeze/thaw cycles to form physical cross-links between polymer chains.[96] Should small molecules like glutaraldehyde be utilized, hydrogels must be rinsed and swollen to ensure all cross-linkers are removed as glutaraldehyde is extremely cytotoxic.[95] PVA hydrogels are known to be elastic, readily swell in aqueous solvents, and are biocompatible. For these reasons, PVA hydrogels have been used clinically for contact lenses, surface coating, and drug delivery.[97]

3.4 Elastomers

As tissues in the body are often dynamic (e.g., blood vessels, muscles, tendons, lungs, skin), they must be allowed to be stretched, strained, and dynamic in culture. Furthermore, it has been shown that mechanical stimulation can play a crucial role in the differentiation of these dynamic tissues. For this reason, synthetic elastic materials have become high in demand. Elastomers are polymers that are viscoelastic, display a very high failure strain, have low Young's moduli, and prior to cross-linking are amorphous.[98] Examples of elastomers in nature include resilin and elastin. In tissue engineering, three classes of biodegradable elastomers have been established: hydrogels, elastin-mimicking peptide chains, and polyhydroxyalkanoates.[99] These polymers exist in coiled formations, and are often cross-linked in their unstressed form. Once cross-linked, upon providing a stress, these coiled chains undergo reversible strains of 5% to 700%.[100] Due to their cross-links, these polymers upon removing the mechanical stimuli return to their unstressed form.

3.5 Polyanhydrides

Polyanhydrides describe a type of biodegradable polymer constructed from repeating monomers connected by anhydride bonds. As the monomeric units and thus properties differ among different types of polyanhydrides, these polymers are subclassified into three main groups: aliphatic, unsaturated, and aromatic.[101] Aliphatic polyanhydrides are soluble in chlorinated hydrocarbons, melt below 100°C, and degrade only weeks after implantation. Unsaturated polyanhydrides include acetylenedicarboxylic acid and 4,4′-stilbendicarboxylic acid, and contain double bonds postpolymerization, which can partake in cross-linking of polymer chains. Due to the double bonds present in the backbone, these polymers also exhibit higher degrees of crystallinity and are insoluble in common organic solvents. The last major class, aromatic polyanhydrides, is extremely hydrophobic, exhibits melting temperatures above 200°C, is insoluble in common organic solvents, and degrades very slowly in vivo.[102] While pure polyanhydrides have limited usage in tissue engineering, they can be blended or copolymerized with aliphatic monomers to increase their degradation time. Polyanhydrides are popular drug delivery materials most notably used in anticancer treatment [carmustine, poly(CPP-SA gliadel wafers)], local anesthetics, neuroactive drugs, and ophthalmic products.

3.6 Polyacetals

Polyacetals are degradable polymers that contain a germinal single carbon flanked by two ether bonds. Similar to polyanhydrides, polyacetals exhibit hydrolytic instability and furthermore have gained popularity as their degradation products are pH-neutral while their degradation is acid-catalyzed.[82] The broader class of polyacetals is subcategorized into either polyacetals or polyketals, where polyacetals specifically describes polymers with one geminal bond with an R group, while polyketals refer to polymers which have both germinal bonds with R groups. Though the most substantial work to date using these materials in biomedical applications has come in the form of drug delivery vehicles, cyclic polyacetals copolymerized with PEG have some success in bone tissue engineering.[103]

3.7 Polyphosphazenes

Polyphosphazenes are a relative novel class of biodegradable polymers characterized by an alternating nitrogen/phosphate backbone with two organic or organometallic side chains ($N = PR^1R^2$). As more than 250 different side groups have been synthesized and because many different types of phosphazene monomers can be polymerized together, the tunability of physical and chemical properties of polyphosphazenes is immense.[104] For stem cell culture and regenerative medicine, polyphosphazenes are unique in that their degradation products are neutral in charge unlike conventional materials like aliphatic polyesters and polyanhydrides whose degradation products are acidic and thus elicit moderate inflammation.[105] Polyanhydrides have found promise in polymer blends, as they are able to neutralize the acidic breakdown products of partner polymers.[106] Of those substituted side groups amino acid esters have displayed great promise as they are both biodegradable, tunable based on the amino acid utilized, and biocompatible. It has been found that these poly[(amino acid ester)phosphazenes] support the growth, adhesion, and spreading of osteoblasts, have been utilized in the repair of periodontal tissue, and show evidence as guide material for peripheral nerve regeneration.[107]

4. SMART POLYMER SYSTEMS FOR 3D STEM CELL CULTURE

The design of biomaterials and tissue engineering scaffolding initially only sought to deliver cells and bioactive agents to a tissue of interest. Scaffolding provided mechanical support, encouraged cell attachment and proliferation, and often was designed to be biodegradable to ensure no remnant synthetic material would remain after tissue replacement. Today, it has been discovered that the native ECM is not simply housing secreted by cells for mechanical support, but instead plays a dynamic role in the communication, differentiation, maturation, and function of cells within a given tissue.[108] Researchers look to "smart" biomaterials capable of responding to external stimuli in order to manipulate cell function, dynamically control scaffold mechanical properties, release biochemical cues, and serve as a conduit for electrical stimulation.

4.1 Thermally Responsive Polymers

As body temperature is relatively constant and universal among persons, many researchers have investigated the use of thermally responsive polymers in both drug delivery and tissue engineering. To understand how temperature affects polymer structure and function, thermoresponsive polymers are classified as either exhibiting a LCST or an upper critical solution temperature (UCST). These values describe a characteristic temperature below or above which a polymer is completely soluble within a given solvent. Thus an LCST polymer in a solvent colder than its LCST would exist as a miscible single-phase liquid and if hotter than its LCST would be immiscible and would precipitate out of solution. This phenomenon can be explained thermodynamically where, as the temperature rises, the entropy of the water is maximized if the polymer is no longer in solution. In the case of a UCST polymer, the relation between temperature and miscibility is reverse.[109] A UCST polymer is thermodynamically governed by the enthalpy of the polymer rather than the entropy of the solvent. For biomedical applications, LCST polymers are favored as increasing the temperature of the polymer–solvent mixture to body temperature can initiate either drug release or polymerization in situ.

4.1.1 *Poly(N-isopropylacrylamide)*

Poly(*N*-isopropylacrylamide) (PNIPAAm) exhibits an LCST near 32°C, just below body temperature and thus has been studied extensively for biomedical applications. At low temperatures, the polymer is able to hydrogen bond with water in solution, while at high temperatures, the entropy of water is favored and the polymer undergoes coil-to-globule transition. This property has been particularly interesting for injectable hydrogel platforms as these materials when cooled can exist as liquids and be mixed with cells and growth factors. After the solution is injected into the body and the temperature of the mixture rises above its LCST, the polymer precipitates, forming interpenetrating polymer networks in situ.

PNIPAAm's unique coil-to-globule transition has been also utilized for controlled drug delivery. Covalently cross-linked PNIPAAm hydrogels below its LCST swell, encapsulating hydrophilic drugs from the solvent (as interactions between the polymer chains and water are favorable) but as the temperature rises to 37°C, the polymer–water interaction becomes unfavorable, chains fold into coils, and the gel rapidly shrinks. Resultantly, water and drug are expelled from the gel matrix and the mechanical modulus of the hydrogel increases dramatically.[110] Modification of the PNIPAAm backbone can alter the transition kinetics and LCST, granting users control over the speed and temperature at which the hydrogel's properties change. PNIPAAm hydrogels like these have been used to control drug delivery, tune cellular phenotype by altering mechanical strain, and as gel actuators converting temperature into mechanical movement. In a simple but interesting use of PNIPAAm, Nakajima et al. (2015) utilized thin films over tissue culture plastic which shrunk when heated to dislodge entire cell sheets. This method has been successfully utilized to generate layers of corneal epithelial cells for human corneal replacement.[111]

4.2 Conductive Polymers

It has been established that electrical stimulation is a critical factor in the differentiation of electrically excitable tissues including bone, skeletal muscle, cardiac, and neuronal tissue.[112,113] These systems exhibit metal-like semiconductive properties while maintaining polymer-like malleability during synthesis. Due to the alternating single and double bonds arranged in planar ringed monomers, these polymers facilitate the transfer of electrons between polymer chains. While electrons in single bonds exist only within σ bonds, double-bond electrons additionally participate in π bonding between weaker, less localized *p* orbitals. Delocalization of these free electrons allows for charge mobility while planar conformation of the double-bond system maximizes π orbital overlapping. Additionally, these polymer chains are stabilized by dopant molecules (usually a simple anion) that neutralize charge. These molecules withdraw or donate electrons to the chain reorganizing native electron clouds and creating localized but loosely bound electrons called polarons or bipolarons. Upon introduction of an electric field, the dopants shift within the polymer chain altering their local environment and allowing for the free flow of electrons.[114]

4.2.1 Polypyrrole

Polypyrrole (PPy) is one of the most well-documented and thoroughly investigated electrically conductive polymers due to its biocompatibility, ease of synthesis and surface modification, and ability to encourage cell attachment and proliferation. Studies involving implantation into the brain, hypodermis tissue, peritoneum, and muscle demonstrate that the material elicits a minimal immune response. PPy has been utilized in biomedical applications as powders, thin films, probes, and cylindrical blood conduits. Upon electrical stimulation, PPy undergoes a reversible oxidation–reduction reaction resulting up to a 35% change in volume[115] and thus has proved to be a powerful tool in controlled pulsatile drug release of both small-molecule drugs, as well as growth factors. Yet despite its many advantages, polypyrrole is not without limitations. While stable at air in room temperature, PPy has exhibited instability in biological environments. This is assumed to be a result of coupling defects in the polymer backbone. In addition, after synthesis, PPy is very difficult to process as it is nonthermoplastic, brittle, rigid, nondegradable, and insoluble in common solvents.[116] These poor mechanical properties make it difficult to mold PPy into complex 3D architectures and hinder its utilization in tissue engineering. To combat these limitations, researchers have attempted to generate polymer–polymer blends with both synthetic [PLGA, PCL, Poly(caprolactone fumarate)] and natural polymers (e.g., HA) to make hydrogels and hard scaffolds with tunable mechanical properties or biodegradable properties.

4.2.2 Polyaniline

Polyaniline (PANI) is second only to PPy as the most utilized electroconductive polymer. Polyaniline can be synthesized either through chemical or electrochemical methods, although electrochemical deposition is often favored as it generates a high purity homogenously distributed film surface coating. Research utilizing PANI in tissue engineering has increased dramatically in the last decade (most notably in cardiac, skeletal, and nerve regeneration) due to its ease of synthesis, low cost, stability, and tunable conductivity based on local microenvironment.[117] PANI has great potential as a smart biomaterial, yet there is great debate concerning its biocompatibility. While minimal inflammation and toxicity have been observed utilizing PANI in short-term in vitro and in vivo studies, several groups have demonstrated deleterious chronic inflammation and fibrous encapsulation of scaffolds implanted for extended periods of time. Debate also concerning human implantation remains, as the degradation product and monomer aniline is known to be highly carcinogenic.[118] For these reasons, significant work has been conducted to determine new synthesis, processing, and fabrication methods to generate safe, biocompatible PANI devices.[119]

4.2.3 PEDOT

Polythiophene derivatives have emerged as an alternative to PPy and PANI and the next generation of electroconductive materials. Of this family of conductive materials, poly(3,4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS) is considered the most successful PTh derivative as it displays a low oxidation potential and has greater thermal and electrochemical stability than either PPy or PANI. This can be attributed to a dioxyalkylene bridging group between the 3 and 4 positions on its heterocyclic ring. The low impedance of PEDOT:PSS results in efficient energy transfer with high signal and low noise. When utilized as a scaffold, it allows for significant cell attachment, proliferation, and signaling of neuronal cells, and even on-demand release of small-molecule drugs.[120–122] PEDOT:PSS has been utilized in the study of the effects of electrical stimulation of embryonic stem cells, coating of neural electrodes, and enhancement of neural stem cell differentiation and proliferation.[123]

4.3 Stimuli Responsive Polymers

4.3.1 Enzymatically Degradable Polymers

Just as the natural ECM can be remodeled, rebuilt, and redesigned by the native cell population, synthetic smart biomaterials can also be modified by cells grown on or within its matrix. This cell-mediated degradation is critical for cell survival and proliferation, as well as whole tissue regeneration.[124] To accomplish this goal, tissue engineers leverage differential expression of proteinases, present in either the local microenvironment of implantation or by a specific cell type. Specifically, the matrix metalloproteinase (MMP) family of enzymes is considered when designing degradable materials. Approximately 30 different MMPs have been identified and both enzymes and their substrates have been well studied and documented. Extensive work by Nagase and Fields (1996)[125] and the Hubbell research group[126] have identified specific peptide sequences which can be utilized for the design of smart enzyme-degradable cell culture platforms. By utilizing enzyme-degradable peptides as cross-linking agents in otherwise nondegradable materials, cells can simultaneously degrade the artificial microenvironment and generate or secrete their own matrix.[94] These MMP-cleavable cross-linking agents have become a cornerstone of most hydrogel platforms.[127] Plasmin, collagenase, and other enzyme-labile sequences have also been incorporated into biopolymer systems to yield biodegradable culture systems (Fig. 19.5A).

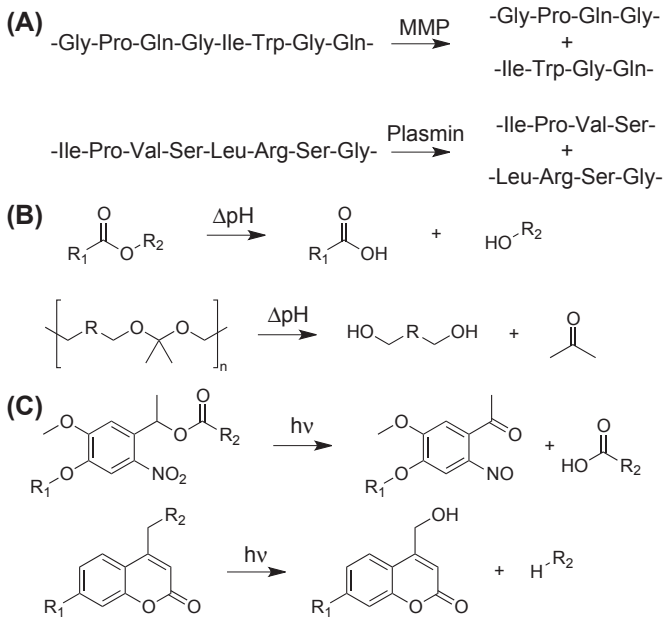

FIGURE 19.5 Stimuli-responsive moieties can be included in polymer backbones and cross-linkers to yield degradable biomaterials. (A) MMP and plasmin produce enzyme-mediated degradation. (B) Polyanhydrides and polyketal derivatives exhibit acid-mediated degradation at pH <7. (C) Photodegradable nitrobenzyl- and coumarin-based cross-linkers exhibit on-demand scission in the presence of UV light.

4.3.2 pH Responsive Polymers

pH responsive polymers have gained the greatest popularity in gene transfection, drug delivery, and cell targeting but have found some success in chronic disease treatment. These polymers and their copolymer blends are designed to alter their physical properties and even degrade in the presence of low pH. The discovery and development of these materials begins with the identification of ionizable polymers with pKa values near pH levels found in the compartment of interest. Weak acids or bases, capable of changing charge are likely candidates and can include compounds containing carboxylic acids, amines, and phosphoric acid functional groups.[128] Examples of these types of monomers include: acrylic acid,[129] maleic anhydride,[130] methacrylic acid,[131] and *N,N*-dimethylaminoethyl methacrylate[132] (Fig. 19.5B) In one study, You et al. (2015) describe the construction of a DMAEMA/HEMA scaffold, capable of inducing swelling in low-pH environments. The study shows that utilization of pH-induced swelling increases oxygen transport, enhanced cell proliferation, and infiltration, ultimately leading to a prohealing in vivo response.[133]

4.3.3 Photoresponsive Polymers

Light is a unique stimulus, allotting exquisite spatiotemporal control and a noninvasive means of initiating change within a given material. Over the past decade, significant research has been conducted to generate chemistries capable of leveraging our understanding of light and optics for tissue engineering purposes.[134] In particular, photolysis, or the use of light to degrade a material or as chemical tether, has been studied for on-demand drug delivery, scaffold fabrication, and the generation of materials with tunable mechanical properties.[135] These linkers include *o*-nitrobenzyl esters, diphenyliodonium carboxylates, and coumarin methyl esters and their derivatives, each of which degrades when subjected to a specific wavelength of light.[134] (Fig. 19.5C). Kinetics of photolysis is well documented and breakdown products and their cytocompatibility are well understood. Utilizing these linkers in the backbone of hydrogel and scaffold constructs allows for site-specific degradation, while utilizing these molecules as linkers to biotherapeutics allow for on-demand drug release.[78,136]

The same spatiotemporal control demonstrated for photodegradation can similarly be utilized for photopolymerization. In particular, light-initiated polymerizations have gained popularity for their ability to encapsulate and deliver cells into complex defects by injection and subsequent transdermal light treatments.[137] Like other radical chain polymerization techniques, photopolymerizable monomers most frequently contain unsaturated vinyl functionalities. By utilizing photoinitiators like riboflavin or Irgacure and the correct wavelength of light, energy is imparted on the initiator and a free radical is generated. This free radical then propagates through unreacted double bonds found in the polymer, producing cross-links between adjacent polymer chains. It is important to note that cell death may occur either due to the generation of free radicals or from UV light exposure (as many photoinitiators require high energy UV light to initiate polymerization). Significant work has been conducted to utilize visible light or IR responsive photoinitiators, as well as safer water-soluble polymers including PEG, chitosan, and gelatin.[29,138,139]

4.3.4 Self-Healing Hydrogels

Self-healing materials and hydrogels are those capable of partially or completely reversible bonding, after mechanical disruption. As bonds readily break and can form again, these interactions often exhibit non-Newtonian shear-thinning properties. In other words, upon shear stress, the material bonds rupture allowing the polymer to act as a liquid. When shear forces subside and the polymer chains are able to interact and reform broken bonds, the material becomes a viscoelastic gel.[140] Several examples have been investigated. Metallogelators are materials utilizing peptides or amino acids that spontaneously complex with metal ions.[141] Work completed by Basak et al. (2014) demonstrates how tyrosine-based amphiphiles spontaneously complex with Ni^{2+} ions. Upon shaking, the materials

became liquid but quickly converted to a viscoelastic state after settling.[142] In an alternative approach, the Burdick group developed "Dock-and-Lock" (DnL) physical hydrogels, utilizing complexation of two self-dimerizing "docking" amino acid chains which spontaneously interact with an anchoring peptide derived from the A-kinase anchoring protein. This shear-thinning material demonstrated the ability to deliver cells into a mock collagenous tissue through an injectable method.[143] These polymer systems have shown great promise as injectable materials, microextrusion 3D printing, and nanofiber materials.[144]

5. CONCLUDING REMARKS

Stem cell-based therapies have the potential to revolutionize modern medicine, providing methods to treat disease, repair damaged tissue, and restore lost organ function. However, the ultimate clinical success of such approaches relies on the ability to expand undifferentiated cells exponentially outside of the body while maintaining pluripotency, yielding large quantities of cells from small isolations. Moreover, being able to transform these undifferentiated patient-derived stem cells efficiently into functional mature lineages of choice is vital for clinical translation. Though strides have been made for both expansion and controlled differentiation, absolute success necessitates a more complete working knowledge of stem cell biology. Critically, we must develop a full understanding of how cellular interpretation of spatiotemporally-presented microenvironmental cues governs lineage commitment. Polymer-based platforms are progressively being used to expand this understanding in vitro, providing simplified culture systems in which to ask fundamental questions about basic biology.

Though we have highlighted several natural and synthetic polymers that have been used to support cell growth, the field will continue to benefit from the development of new polymer systems. The advent of high-throughput fabrication enables vast multicomponent copolymer arrays to be created reproducibly and inexpensively, enabling cell–material interactions to be teased out with greater detail than ever before. Additionally, new monomer types and polymer chemistries continue to expand the library of species that could be useful to probe and direct stem cell fate. Just as cues are presented to cells in the native tissue environment, polymer-based platforms capable of re-creating this dynamic heterogeneity are in great need. Programmable systems offering complete spatial and temporal variation in the biochemical and biophysical aspects of the culture platform would provide researchers new dimensions of cellular control.[145,146] We expect that these advances in polymer design and development will be critical in helping stem cell therapies reach their full potential.

ABBREVIATIONS AND ACRONYMS

CS Chondroitin sulfate
dECM Decellularized extracellular matrix
DnL Dock-and-Lock
ECM Extracellular matrix
EHS Engelbreth-Holm-Swarm
ELPs Elastin-like polypeptides
Fmoc 9-Fluorenylmethoxycarbonyl
HA Hyaluronic acid
Hyp Hydroxyproline
LCST Lower critical solution temperature
MMP Matrix metalloproteinase
PANI Polyaniline
PAs Peptide amphiphiles
PCL Polycaprolactone
PEDOT Poly(3,4-ethylenedioxythiophene)
PEG Poly(ethylene glycol)
PGA Polyglycolide
PLA Polylactic acid
PLGA Poly(lactic-co-glycolic acid)
PNIPAAm Poly(*N*-isopropylacrylamide)
PPY Polypyrrole
PSS Polystyrene sulfonate
PVA Poly(vinyl alcohol)
UCST Upper critical solution temperature

Glossary

Bioactive The quality of a substance to impart an effect (beneficial or adverse) on a biological organism or process. Although bioactivity of a treatment is dose dependent, bioactivity in a biomaterial context is most often a measure of the intrinsic efficacy of the substance to impart a physiological response.

Bioconjugation The process or strategy to bond two molecules or chemicals together, one of which is present in or generated by a biological organism.

Biodegradable The material quality of being chemically dissolved by a process involved in biological function.

Bioinert The quality of not interacting with biological organisms. Bioinert materials exert minimal or no effect on biological function.

Cross-linking The strategy of joining two or more polymer chains together through one or more chemical bonds. The type of chemical bonds involved can include covalent, ionic, van der Waals forces, as well as other physical interactions.

Crystallinity The degree to which a polymer network demonstrates partial alignment of its molecular chains. Polymer crystallinity in solid samples is dictated by the relative ratio of regions of amorphous disarrangement or aligned sheets of polymer chains. As polymers are rarely completely amorphous or crystalline, most are considered semicrystalline.

Extracellular matrix (ECM) The material substance secreted by cells into their environment, which provides structural and biochemical support to surrounding cells. Generally, ECM is composed of a mixture of proteins, polysaccharides, and glycosaminoglycans.

Glass transition temperature (T_g) The temperature above which a semicrystalline material undergoes a transformation from a relatively stiff brittle state to that of an amorphous viscoelastic solid.

Hydrogel An interconnected network of hydrophilic polymer chains which as a collective exceeds solubility limitations in water. As a consequence of their hydrophilic nature, hydrogels demonstrate large swelling ratios, are absorbent, and consist mostly of water by mass. Hydrogels have found extensive use as implantable devices, cell culture platforms, contact lenses, and medical wound dressings.

Immunogenic The ability of a material or substance to elicit an immune response.

Macromolecule A single large molecule often generated by the covalent bonding of smaller subunits. In biochemistry, the four main categories of biomacromolecules include proteins, nucleic acids, fats, and carbohydrates. Synthetic examples of macromolecules include synthetic polymers and carbon-based molecules like nanotubes and graphene.

Matrix metalloproteinase (MMP) A family of zinc-containing calcium-dependent endopeptidases that generally cleave extracellular matrix proteins. MMPs are involved in cell migration, signal transduction, apoptosis, proliferation, migration, differentiation, and angiogenesis. Examples of MMPs include collagenases, elastases, and gelatinases.

Mechanical modulus A general term used to describe the stiffness of a given material. Measurements of moduli might be reported as Young's/elastic, shear, or bulk moduli, denoting a material's resistance to compression, shear, and uniform hydrostatic pressure, respectively.

Physical cross-linking (entanglement) The physical restriction imparted on a polymer chain as a consequence of its intertwining within a polymer network. While physical cross-links do not involve specific moieties on a polymer chain, interpenetrating networks can become so large that they precipitate out of solution and become a semisolid hydrogel.

Polydispersity index (for polymers) An index used to describe the relative distribution of sizes/molecular weights within a sample of polymers. Often, these populations consist of a single type of polymers with varying chain lengths. PDI is defined as the weight average molecular weight divided by the number average molecular weight of a polymer species and typically ranges from 1 to 30 where 1 would describe a sample containing a single uniform (homogenously sized) polymer population, and a value of 30+ would describe a nonuniform (heterogeneously sized) polymers. PDI is typically most affected by the manufacturing or polymerization process.

Polymer A term used to describe a class of macromolecules composed of repeating monomeric subunits. The process/strategy of chemical bonding of these individual repeating units is known as polymerization.

Self-assembly The process in which molecules or components spontaneously become ordered. Self-assembly is maintained by reversible, noncovalent bonds in response to favorable local intermolecular, intramolecular, and solvent interactions.

Shear-thinning A rheological phenomenon in which a fluid that becomes less viscous when experiencing shear stress. Shear-thinning materials are also called non-Newtonian pseudoplastic fluids. Examples include blood and paint.

Strain The material deformation associated with a given mechanical load. Although strain can occur as a result of shear, compression, or hydrostatic pressure, the simplest measure (engineering strain) is defined as the change in length divided by the original length of a material.

Tensile strength A critical tension (pulling) force required to permanently deform a given material. Tensile strength is most often reported as yield strength (force required to elicit a permanent deformation exceeding 0.02% of the initial dimension), ultimate strength (maximum stress tolerable by a material when under tension), or fracture strength (stress associated with fracture).

Viscoelasticity The property of a material that demonstrates both viscous fluid components (permanent energy-consuming deformation over time) and elastic solid components (reversible energy-conserving deformation). Viscoelastic materials demonstrate three unique properties: differences in stress and strain depending on the history of mechanical deformation (hysteresis), decreasing stress given a constant strain (stress relaxation), and increasing strain given a constant stress (creep). Nearly all biological materials demonstrate some degree of viscoelasticity.

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