Chapter 13

Polymers for Bioprinting


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ABSTRACT

Bioprinting is a process of precisely designed scaffolds using three-dimensional printing technologies for functional tissue engineering utilizing cell-laden biomaterials as bioink. A range of polymers can be used as bioink to stimulate favorable cellular interactions, leading to enhanced cell motility, proliferation, and subsequent differentiation. Both natural and synthetic polymers have been considered for various bioprinting applications, each with a corresponding set of advantages and limitations. Natural polymers more aptly mimic the native extracellular matrix, leading to more favorable cellular responses, while synthetic polymers can be more easily tailored for more efficient printing. Because many of these bioink materials are rooted in traditional tissue engineering scaffold design, bioprinting optimization remains a challenge; however, emerging trends in bioink development have begun to circumvent these issues, providing bioprinting research with a very promising future in regenerative medicine. Further investigation into the interplay of polymer type and fabrication technique will help to formulate new polymer bioinks that can expedite the process from printing to implantation.

Keywords: bioprinting; natural polymers; synthetic polymers; polymer hybrids; polymer blends; tissue engineering; 3D printing

1 INTRODUCTION

Bioprinting is a process of precisely designed scaffolds using three-dimensional (3D) printing technologies for functional organ engineering. Due to the pressing need for functional organ engineering, precisely designed scaffolds for tissue repair and organ replacement are needed. The emergence of nano- and microscale printing technologies resulted in the development of 3D-printed scaffolds consisting of spatially controlled cell patterns that may be loaded
with appropriate biological moieties to control or direct cell fate [1–4]. The rationale for such significant control over spatially driven design is to better coordinate cellular arrangements into tissues and organs of interest and therefore lead to the successful production of functional and implantable constructs. Bioprinting remains in its early stages of development but continues to gain popularity amongst regenerative medicine researchers due to its immense potential in the field (Fig. 13.1). A steady increase in the number of publications and citations in the area of bioprinting indicate its huge potential in biomedical applications including tissue engineering, drug development, and organ-on-chip platforms. While challenges exist to maintain the intended shape and cell distribution of the construct over time, researchers have employed a variety of novel methods and technologies to improve upon the bioprinting process [5–8].

A vital aspect and bottleneck to the design and implementation of a bioprinting system is the consideration of a bioink. The bioink mainly comprises of a polymer matrix loaded with cells and bioactive signals [9]. By controlling the physical and chemical properties of the extracellular matrix (ECM), cell behavior can be regulated to accelerate tissue integration and functional recovery. Thus, it is important to carefully select polymers for bioprinting to support and enhance tissue regeneration in a temporospatial manner [10]. A range of polymers can be used as a bioink to provide tunability to stimulate favorable cellular interactions, leading to enhanced cell motility, proliferation, and subsequent differentiation (Fig. 13.2). In order to mimic native tissue microenvironment and to drive regeneration of tissue, many researchers are focusing on natural polymers to fabricate 3D printed scaffolds [11]. This is mainly due to the chemical and structural similarities of natural polymers to native ECM. Some of the natural polymers that are currently explored for bioprinting include collagen/gelatin, alginate, fibrin, hyaluronan, and dextran. Synthetic polymers, however, can be more easily tailored for a given application by optimizing mechanical properties, degradation rates, or functionalized with a variety of bioactive factors [12]. Some of the synthetic polymers that are currently used in bioprinting include poly(ethylene glycol) (PEG), poly(lactide-co-glycolide) (PLGA), poly(ε-caprolactone) (PCL), and poly(lactic acid) (PLLA) (Fig. 13.2). Blends of natural and synthetic polymers can result in a combination of benefits in an attempt to enhance and tailor cellular responses within the 3D fabricated scaffolds. For example, synthetic polymers can be copolymerized with enzymatic degradation sites found in natural polymers to enhance cell migration and aggregation within the scaffold [13]. These polymer combinations used in conjunction with the spatial resolution of many bioprinting technologies enable a more efficacious tissue and organ regeneration process.

FIGURE 13.2 Chart diagramming natural (red) and synthetic (blue) polymer distributions for use as bioinks compiled from relevant literature. Hybrid systems split into polymer constituents for consideration.

FIGURE 13.1 Publication trends over the past decade demonstrating a significant rise in the field of bioprinting. (a) Trends in number of publications, and (b) number of citations for articles pertaining to bioprinting (Data obtained on June 2015 from ISI Web of Science).
In this chapter, we focus on various natural and synthetic polymer systems for bioprinting. We will discuss some of the important physical and chemical properties of polymers that play a crucial role in the bioprinting process. Different types of polymers, their physical, chemical, and biological properties that require optimization for specific bioprinting methods will also be discussed. Specifically, polymer properties as they apply to synthetic, natural, and hybrid systems, will be discussed. Emerging trends, such as polymer-nanocomposite bioinks, growth factor incorporation, and multinozzle polymer systems, along with future directions in bioink research, are highlighted. Additionally, some of the most critical challenges associated with the selection, fabrication, and evaluation of polymeric systems for bioprinting are emphasized.

2 POLYMER PROPERTIES FOR BIOPRINTING

A true material science approach toward bioink design is essential for bioink success. This material design approach is required to understand how polymer characteristics influence printing efficacy and cytocompatibility. Because these bioinks will be subjected to the printing process and then expected to maintain structural integrity, there is an inherent complexity of bioink production. Properties like viscoelasticity of polymer bioinks, which affects printed structure outcome, or polymer behavior in solution, which impacts printing ability, are common design criteria. Similarly, polymers demonstrating creep and stress relaxation can alter material shape and lead to impeded functionality. Other considerations like shelf-life and cost of polymer bioinks are also important determinants of material choice.

Many of the important properties relevant for bioprinting are illustrated in Fig. 13.3. Additionally, while the availability of natural polymers is more limited than synthetics, the majority of research on bioinks has focused on naturally derived materials. The challenge to simultaneously optimize polymer–polymer interactions as well as polymer–cell interactions while maintaining printability persists in current bioprinting research. A need exists to develop bioink-specific materials as opposed to investigators transposing traditional tissue engineering biomaterials into forced bioprinting roles.

**FIGURE 13.3** Polymer properties considered for bioprinting applications. (a) Hydration of a polymer system enables nutrient and waste transport to encapsulated cells deep within a printed construct. (b) Degradation mechanism can be modified to influence cellular migration and tissue regeneration. (c) Viscous solutions can better suspend and shield cells from shear forces within the nozzle, yet are more likely to obstruct flow, while low viscosity solutions can avoid clogging however can suffer from cell settling. (d) Cross-linking mechanism is dependent on polymer type, cell viability considerations, and desired construct properties as cross-links can exist permanently or reversibly. (e) Cell adhesion influences cell fate and can be controlled through polymer type or processing. (f) Stability of a printed material is vital to the success as it should mimic native tissue mechanically as well as maintain its shape to organize cell growth.
2.1 Cross-Linking Mechanism

The type of polymer selected for bioprinting mainly depends on the type of cross-linking techniques employed to generate 3D networks. Various cross-linking mechanisms to obtain structurally stable polymeric network include photo-, ion-, electrostatic-, pH-, and temperature-based cross-linking mechanisms. Care must be taken when choosing the cross-linking method, as the viability of the encapsulated cells will significantly depend on the environmental conditions such as heat or pH. Similarly, the cross-linking technique influences construct outcomes with considerations of gelation via reversible or irreversible processes. Specifically, thermal gelation mechanisms result in constructs with a temporary shape that can vary with temperature, while chemical gelation can provide a permanent shape with a lower chance of deformation over time. The trade-off in many instances, however, is that the time required for chemical-dependent solidification exceeds that of temperature-dependent gelation. For example, a variety of polymers can be modified chemically via introduction of acrylate or methacrylate groups, which can subsequently be photocrosslinked in the presence of a photoinitiator (PI) to form a covalently cross-linked network [15,16]. For example, reacting poly(ethylene glycol) (PEG) with methacryloyl chloride in the presence of triethylamine and modifying gelatin with unsaturated methacrylamide side groups yield photocrosslinkable poly(ethylene glycol) dimethacrylate (PEGDMA) and gelatin methacrylamide, respectively [17,18]. Physical cross-links can be similarly induced ionically via an additional cation solution (e.g., Ca$^{2+}$) to produce natural polymer-based networks [4,5,16]. For example, sodium alginate solution gels when in contact with Ca$^{2+}$, a divalent cation, tethers neighboring G-blocks of alginate chains, forming a reversible electrostatic bridge. The degree of gelation can be tuned by adjusting the concentration of CaCl$_2$ solution [19–22]. Thermoresponsive gelation of gelatin can also be employed in bioprinting since it aids in retaining the shape of printed constructs. However, native gelatin has not been used alone for bioprinting because its reversible sol–gel transition (i.e., upper critical solution temperature (UCST) material behavior) poses difficulties in optimizing printing temperature and viscosity. These cross-links can then influence the mechanical strength of the fabricated tissue as well as influence cellular responses of encapsulated stem cells through a more efficient distribution of mechanical stresses or reducing migration [23–25]. The resulting networked materials can display thixotropic properties where the stress of extrusion induces a quasi-liquid state until exiting the nozzle, where it displays a solid-like behavior once more [5]. Depending on the cross-linking method (e.g., ionic, photonic, or electrostatic) and the polymer concentration, printing efficiency can be significantly impacted. Lastly, many extrudable polymers maintain their structural integrity by using a high curing temperature or solvents toxic to cells for polymerization [26]. These conditions (e.g., extreme temperature) could be too harsh for cell survival. As a result, not all polymers used for 3D printing, where cells are seeded after scaffold fabrication, can be used for bioprinting. For example, heat treatment at 100°C is necessary to dry and maintain structural integrity of printed starch constructs. This extreme temperature prevents the use of starch blend for bioprinting [27].

2.2 Viscosity

Determination of the polymer structure and its rheological properties are also important for smooth extrusion of ink in contact of high shear rate (≈20–200 s$^{-1}$) and shape retention [28]. The most critical location during extrusion is the dispensing tip where clogging and fracture can occur if the viscosity is too high while an inadequate viscosity will impart shear forces on the cells, resulting in death [29]. The printed polymer should demonstrate multiple physical phases: first like a fluid within the printer and then a solid once dispensed. This will maintain the extruded filament-like shape. Polymers with shear thinning behavior promote this solidification process since viscosity will increase significantly as shearing wanes after extrusion [26,28]. Many bioprinted polymers have been shown to exhibit shear thinning behavior. For example, silk fibroin molecules align and the friction resistance between adjacent fibers decreases as shear rate increases [30]. These shear-thinning capabilities reduce nozzle-clogging effects and provide researchers with an alternative over the use of low-viscosity polymer solutions. Higher viscosity polymer bioinks from increased cross-linking density also reduce the likelihood of nozzle obstruction from cell aggregates as cells are better suspended in the bioink; however, printing time and risks of cell death or gel fracture increases with viscosity among other challenges to this approach [5,10]. Regarding the rheological properties, one challenge posed by natural polymers is variability of viscosity from batch to batch, affecting optimal printing parameters including nozzle size, shear rate, and pressure needed [28]. Particularly important is the ability to shield the biological components from the forces associated with printing. Cell suspensions surrounded by a more gel-like material will be more likely to retain viability as opposed to more fluid-like bioinks. The use of semi-interpenetrating polymer networks (semi-IPN) can produce a hydrogel with mechanical stability as well as being sufficiently hydrated to enable nutrient transfer and construct shape due to the presence of a secondary uncross-linked polymer web. In some cases, charged domains on the cross-linked network can provide electrostatic repulsion for increased hydration and strength, although future research is needed for the practicality of such a bioink.
2.3 Hydration Properties

One property that can greatly alter the viscosity of the bioink is hydration of the material. Hydration and porosity provide control over mechanical strength, tunable viscoelasticity and in the design of optimized pressure through the micronozzle during the fabrication process. Apart from that, oxygen and nutrient transport to the laden cells within the hydrogel network are prerequisites for any successful engineered tissue. Many natural polymers and hydrophilic synthetic polymers, such as PLGA and PEG, are finding expanded applicability in designing complex tissue structures like blood vessels, where on one hand the fluidity of the material allows for easy fabrication while hydration enables the material to successfully mimic natural tissue. There is a trade-off, however, between porosity or hydration and mechanical strength. With increased pore size, the modulus of the hydrogel decreases because of increased water-to-hydrogel contact, and water acts as a plasticizer within the polymer chains. Thermo-responsive hydrogels in particular are known to have more aqueous uptake and therefore slower deswelling kinetics, which could be important for applications in which the hydrogel is heated or cooled upon extrusion.

2.4 Biological Interaction Properties

Physical properties of polymers, such as hydrophilicity and surface energy, influence cellular behavior and are important parameters in determining the applicability of polymers for generating specific tissues. For example, polymers with cell adhesive site are required to enhance survival and proliferation of adhesive types of cells such as osteoblasts. However, chondrocytes would prefer to be encapsulated in hydrogels with minimum cell adhesion sites. Polymer matrices that interact on similar length scales to mechano-receptors present on cell membranes have also been documented to improve differentiation of seeded stem cells. For example, collagen and gelatin hold Arg–Gly–Asp (RGD) sequences, which promote cell adhesion via integrin receptors [31,32]. Polymers without cell binding moieties, such as synthetic polymers and some natural polymers like gelatin, may prevent cell adhesion and proliferation. Apoptosis could be induced as a result of the lack of cell adhesion, which is termed “anoikis.” This drawback could be overcome by adding cell adhesive molecules, for example, by blending synthetic polymers with natural polymers and blending alginate with gelatin [33–35]. It should be noted that many synthetic polymers that are widely used for tissue engineering, such as PCL and PLLA, have been shown to exhibit good cytocompatibility and promote cell adhesion and proliferation even in the absence of cell-binding moieties [34,36,37]. Nevertheless, their biological properties could be further enhanced by an introduction of cell adhesive molecules.

2.5 Mechanical Properties

The mechanical functions of natural tissues are vital in the replication process by any proposed biomaterial considered for bioprinting. Invariably these natural tissues are composed of a stiff acellular environment surrounded by softer cellular matrices. Several cell-laden thermoplastic polymers have been explored to construct the well-organized acellular content with a range of rigidities and desired mechanical properties. Along these same lines, the mechanical properties of a chosen polymer are heavily reliant on chemical composition. Consequently, there is an intimate relationship between biomaterial robustness and molecular arrangement of the included polymer. Therefore, an understanding of the factors that influence polymer mechanical behavior is required to effectively design a bioprinting system. By varying cross-link density and therefore the tensile modulus of the hydrogel, stem cell interactions with the environment, through actin-mediated pathways for example, can be controlled. This leads to control over differentiation and subsequent formation of tissue.

2.6 Polymer Chain Considerations

Aside from those polymer properties previously described, there are multiple interactions specific to the polymer backbone that alter response to the environment. One such interplay stems from varying temperature. Because of the thermal sensitivity of polymers through chain mobility, temperature control during the bioprinting process could be required, especially for those with low glass transition temperatures ($T_g$). Solvent interactions also affect bulk material behavior; for example, polymer hydrophilicity and concentration can significantly impact hydrogel swelling. The presence of water not only affects mechanical properties of the material, but also transport of nutrients and metabolic waste products within the polymer matrix, which influences cell proliferation. Another crucial consideration of printed polymers is the mechanism and rate of degradation. Hydrolyzable domains, like in polyanhydrides, polylethesters, polyamides, and polysters, enable variable degradation rates of synthetic polymer constructs. Enzymatically cleavable sites, like those found in naturally based polymers, are important to consider particularly for cell migration through a bioprinted hydrogel as biological agents in the surrounding environment cause localized mass loss of the material through the release of specific enzymes. Once these linkage sites have been cleaved, the polymer can incur mass loss, either at the surface of the bulk material or internally, depending on the relative rates of water penetration and chain scission.

2.7 Fabrication Techniques

A multitude of microscale technologies have emerged that employ preprocessing of polymers to generate functional
strands capable of cross-linking [16,38]. Some of the fabrication methods include drop-on-demand bioprinting with inkjet nozzles utilizing thermal or piezoelectric technology to generate displacement forces, stereolithography, or laser-assisted printing [16,39,40]. Due to the variation between printing methods and physiological environments associated with various applications, the development of tailored bioinks is necessary to ensure tissue and organ functionality. Individual building blocks comprised of a cell-laden biomaterial are one such microscale technology to materialize. Hydrogel droplets, or “tissue spheroids,” containing large amounts of cells can fuse together over time, resulting in a highly controllable tissue construct that does not require a scaffold [8]. Multiple technologies have also emerged to fabricate constructs with a combination of polymer inks as well as cell types. These methodologies are particularly useful for therapies like osteochondral regeneration where the tissue adjoins multiple cell types comprising regions of different mechanical strength and stiffness [41]. As the complexity of new printing technologies rises, so too will the intricacy of bioink interactions. Interpolymer interactions between separate bioinks could result in better construct stability or enhanced cellular behavior.

3 NATURAL POLYMERS FOR BIOPRINTING

The selection of polymers is critically important for the bioprinting process. Natural polymers can avoid coarse fabrication conditions like temperature and organic solvents, thereby allowing the printing of cells and bioactive components [28]. However, there are three key challenges associated with printing natural polymers. First, innate characteristics, such as viscosity, can fluctuate over a group of natural polymers, making printing reproducible scaffolds with a precise structure a challenge. Also, the presence of moisture can be inconsistent in polymer groups, varying pressure needed to print the polymer inks. The other challenges include strut solidification and the interplay of charged components between polymer chains and fluid if the polymer ink is plotted into a solution. Solidification in air requires the polymer to dry at an optimum rate to support and merge to subsequent printed layers. If printed into liquid media, density of the tissue adjoins multiple cell types comprising regions of different mechanical strength and stiffness [41]. As the complexity of new printing technologies rises, so too will the intricacy of bioink interactions. Interpolymer interactions between separate bioinks could result in better construct stability or enhanced cellular behavior.

3.1 Natural Polymers for Bioprinting

Of the bioinks currently established for research, many stem from naturally derived polymers. The following polymers alone provide significant cytocompatibility through simulating a natural environment for tissue remodeling. A multitude of cross-linking methods are capable of generating a solidified construct with varying degrees of mechanical integrity.

3.1.1 Collagen/Gelatin

Collagen contains a large quantity of glycine, proline, and hydroxyproline residues, and contains a small amount of aromatic and sulfur-containing amino acids. Pyrrolidine (i.e., proline and hydroxyproline) is responsible for the stabilization of the tertiary super-helix structure through steric hindrance. Collagen significantly constitutes the ECM and participates in numerous physiological interactions. Its contacts with cells modulate cell adhesion, migration, proliferation, and differentiation [32]. As a result, collagen is widely used to fabricate tissue-engineering scaffolds. However, the inferior mechanical integrity limits its application in bioprinting [42].

Losing the secondary structure as well as tertiary and primary structures found in collagen results in the denaturalized form of gelatin. As a result, the helical arrangement of collagen is lost to the randomness present in gelatin. Gelatin is cooled at temperatures above 40°C in aqueous environments; and it reversibly forms an alpha helix when the solution is cooled to below 30°C. At diluted concentrations, chain mobility can produce intramolecular bonds. When the concentration increases to above 1%, chain association and three-dimensional networks are induced. The manner and extension of this reversible fold of the triple-helix structure appears to depend on solvent, concentration, and temperature [43]. Gelatin is widely used for tissue engineering applications since it has almost identical composition to collagen, the main component of natural ECM. Specifically, it holds RGD sequences, which promotes cell adhesion via integrin receptors. In addition, since gelatin is a denatured polymer, concerns of immunogenicity and pathogen transmission associated with collagen can be circumvented.

Thermoresponsive transition of gelatin could be employed in bioprinting since it aids in structure maintenance upon printing. However, native gelatin has not been used alone for bioprinting because this temperature-dependent, but reversible sol–gel transition (i.e., UCST material behavior), poses difficulties in optimizing printing temperature and viscosity. This shortcoming can be overcome by chemical modification and/or combining gelatin with other polymers. For example, gelatin can be modified with unsaturated
methacrylamide side groups to yield a photosensitive gelatin derivative, gelatin methacrylamide [17]. A stable cross-linked scaffold can be obtained by exposing to UV radiation in the presence of a PI. After photocrosslinking of gelatin chains through methacrylamide double bonds, thermal gelation of physical crosslinks are no longer temperature responsive (i.e., become irreversible) [44].

In a similar approach, Billiet et al. reported bioprinting of cell-laden gelatin methacrylamide using a pneumatic-based bioplotter [17]. They observed that the types of PI have profound effects on cell viability. In this study, water soluble halogen-free azo initiator (2,2′-azobis[2-methyl-N-(2-hydroxyethyl)propionamide], also known as VA-086 (Wako Chemicals, USA)) gave rise to enhanced hepatocarcinoma cell viability (<97%) compared to the conventional alpha-hydroxy ketone-based PI (2959 (2-hydroxy-1-[4-(2-hydroxyethoxy) phenyl]-2-methyl-1-propanone, also known as Irgacure). The prepolymer solution containing gelatin methacrylamide showed shear thinning behavior that is preferable for bioprinting. The scaffolds with a 100% interconnected pore network could be produced using 10–20% w/v% gelatin methacrylamide. Another research group reported impaired construct formation with gelatin methacrylamide less than 20 w/v% due to severe viscosity issues caused by difficult temperature control. They solved this problem by blending gelatin methacrylamide with hyaluronic acid to increase the solution viscosity [44]. On the other hand, Billiet et al. were able to produce gelatin-only scaffolds by varying gelatin acrylamide concentrations [17]. This was achieved by optimizing multiple printing parameters including concentration, temperature, pressure, nozzle type and diameter, and plotting speed. Also, device adaptations were necessary to ensure homogenous plotting temperature and enable the cooling of the platform to a temperature far below the gelting point.

Similar to gelatin methacrylamide, gelatin methacrylate (GelMA) is electrostatically charged and has intrinsic adhesive properties owing to its uncured acrylate groups. Cell-laden gelatin methacrylate was bioprinted followed by photocrosslinking under UV light [45]. Printability declined with reducing UV exposure time, GelMA concentration, and increased cell density. Printed HepG2 cells could be preserved at viability levels higher than 80%. In addition, cell proliferation in printed constructs was found to be higher than control hydrogel blocks. This could be attributed to easier access to nutrients in precisely designed bioprinted scaffolds.

Double chemical functionalization could further enhance control over bioprinting. For example, methacrylation and acetylation of gelatin allowed control over solution viscosity, gelling behaviors, and photochemical cross-linking simultaneously [31]. Particularly, the degree of methacrylation determined the mechanical properties of cross-linked constructs while additional acetylation influenced rheological properties of the gelatin solution. The degree of methacrylation could be adjusted by molar excess of methacrylic anhydride during the methacrylation process. Highly methacrylated gelatin made from 10-fold molar excess had low viscosities within inkjet-printable range (3.3 ± 0.5 mPa•s, 37°C) and resulted in cross-linked constructs with high storage moduli G′ (15.2 ± 6.4 kPa). Twofold molar excess was suitable for the preparation of soft hydrogels, but its solution was highly viscous and could result in nozzle clogging. The viscosity could be reduced by additional acetylation of GelMA. In this manner, a two-fold functionalized gelation solution could be inkjet printed and could result in a soft gel with a low degree of cross-linking. It should be noted that unmodified gelatin and GelMA with low degrees of methacrylation have high viscosity even above their melting temperature and gel at room temperature. Consequently, they are prone to clogging nozzles. The printed constructs were found to be cyto compatible. Porcine chondrocytes printed with two-fold-modified gelatin had high viability and normal functionality.

3.1.2 Alginate

Alginate is an anionic polysaccharide obtained from brown seaweed. The raw material extracted from seaweed is known as sodium alginate. The terms alginate and sodium alginate are often used interchangeably. Alginate is a linear block copolymer composed of β-1,4-d-mannuronic acid monomers (M-blocks) in sequence with α-l-guluronic acid blocks (G-blocks), and intermixed M and G domains. Alginate solutions gel with divalent cations due to ionic bridge formation between G-blocks [19,21,22,46]. Structural similarities to natural ECM, an excellent biocompatibility, viscosity, and the ease of gelation that takes place at room temperature make it attractive for bioprinting [19,21,22,46].

Nakamura et al. printed 3D alginate cell-laden constructs using an inkjet printer [33]. Alginate solution was ejected onto a calcium chloride (CaCl₂) solution and each droplet formed a homogenous gel bead. The beads then fused together forming gel fibers and finally 3D gel constructs. The printed constructs were found to have good mechanical integrity and biocompatibility. However, since alginate does not have cell-binding moieties, such as RGD sequences, it may prevent cell adhesion and proliferation. Apoptosis could be induced due to the lack of cell adhesion, which is termed “anoikis.” This drawback could be overcome by adding cell adhesive molecules, for example, by blending alginate with gelatin or other polymers with cell adhesive sites.

In addition, alginate biomaterials encapsulating endothelial cells were printed using a multinozzle deposition system [47]. Suitable fabrication parameters were found to be 1.5% w/v alginate and 0.5% w/v CaCl₂. The elastic modulus of printed scaffolds increased from day 0 to day 1, and then gradually decreased over time. This could be
attributed to continued alginate cross-linking by ions present in the cell culture media. The ions diffused and interchanged within scaffolds, allowing alginate chains to detach from the main cross-linked constructs and diffuse out. Eventually, the structures degraded and lost their mechanical integrity over time. The viability of encapsulated cells ranged between 76% and 83% for printing shear stresses of 100–1150 kPa, respectively [47]. Similarly, a solution with 1% w/v alginate and 1% CaCl$_2$ was successfully printed using a multinozzle system [46]. However, dragging between printed layers caused by viscous fraction made the pattern slant, hindering accurate shaping of structures. The possible solutions to this problem are optimizing gelling duration and extent, and adopting a feedback control system of printing speed using a vision system that can monitor the printed construct in real time [46].

There are two main challenges associated with bioprinting of alginate. First, it is difficult to print 3D cell-laden alginate scaffolds with completely interconnected pores due to the difficulty in controlling the gelation process. Second, the thickness of constructs that can be printed is limited because alginate has favorable interactions with water and minimal viscosity hindering thick structures printing. Specifically, since alginate is highly soluble in aqueous solution, dispensing alginate directly in CaCl$_2$ solution can weaken the constructs. To circumvent these challenges, Ahn et al. proposed a new printing system consisting of a dispensing method and an aerosol-spraying method [19]. A cell-laden alginate solution was printed using a dispensing system followed by an aerosol spray of CaCl$_2$. After printing, the construct was immersed in CaCl$_2$ solution for a second curing. Before an aerosol spray, viability of encapsulated preosteoblast cells (MC3T3-E1) was as high as 97%. With increasing weight fractions of CaCl$_2$, cell viability decreased. To further incorporate bioactive clues, researchers have incorporated proteins/drug-loaded microparticles within the printed alginate scaffold [48]. For example, bone morphogenetic protein 2 (BMP-2)-loaded gelatin microparticles were embedded in cell-laden alginate and showed osteogenicity in vivo [48]. The encapsulation of BMP-2 within the gelatin microparticles results in sustained release of the protein. It is expected that by incorporating such bioactive clues cellular process can be controlled.

3.1.3 Fibrin

Fibrin is formed by the interaction between fibrinogen and thrombin, the mechanism known for blood coagulation. It is also a component of natural ECM. Fibrinogen is a glycoprotein consisting of multiple pairs of polypeptide chains: $\alpha$, $\beta$, and $\gamma$. It contains a cell-signaling domain including protease degradation and cell adhesion motifs. To form fibrin gel, thrombin cleaves $\alpha$ and $\beta$ chains to fibrinopeptide A and B [33,49]. Then, these fibrin monomers spontaneously polymerize to form protofibrils, which associate laterally to form fibrin fibers. Finally, fibrin fibers associate to form fibrin gel [33,49]. Due to the presence of cell adhesion motifs and the ease of gelation, fibrin has a potential for bioprinting. Cells were found to adhere and proliferate well in cell-laden printed fibrin scaffolds [33]. In comparison to alginate-only gel-laden constructs, fibrin has an advantage in terms of cytocompatibility. This is mainly due to the presence of cell adhesion moieties within the fibrin structure. However, fibrin gel, formed by an inkjet printer, was shown to be soft and fragile, and has difficulty in maintaining its 3D structure [33]. Some of these drawbacks can be overcome by combining fibrin with different natural and synthetic polymers. Although fibrin-based materials are promising, very limited work has been reported using fibrin-based bioink.

3.2 Cell-Laden Polymer Blends for Bioprinting

As opposed to a single naturally derived polymer for use as a bioink, multiple polymers may be blended together to improve printing efficacy or construct performance. We will discuss these polymer systems in particular in the following section.

3.2.1 Fibrin/Collagen

Fibrin and collagen have been used for bioprinting. Both of them are ECM components and have excellent biocompatibility. Fibrin/collagen blend solutions containing amniotic-fluid-derived stem cells and bone-marrow-derived mesenchymal stem cells were bioprinted onto full-thickness skin wounds [50]. Gelation was achieved by alternate printing of fibrin/collagen layer and thrombin. Migration and integration of cells into regenerated tissues were not observed, suggesting that the mobility of printed cells was limited due to fibrin/collagen struts. Fibrin and collagen contain cell adhesive motifs that might prevent the cell from being mobile. This could potentially limit therapeutic success. In addition, collagen naturally contracts when cross-linked, which could be detrimental to the healing process, resulting in fibrosis and scarring. In another study, bioprinting of collagen and fibrin gel loaded with VEGF have been used for culturing neural stem cells. In this study, collagen type I was chosen as a main scaffold material, while fibrin gel was used for VEGF delivery and to promote migration and proliferation of embedded cells. With combined biological effects of collagen and VEGF release, neural stem cells showed signs of differentiation after 2 days.

3.2.2 Gelatin/Alginate

Gelatin/alginate blend combines thermoresponsive qualities of gelatin with the chemical cross-linking ability of alginate [29,51]. The main role of gelatin is to alter the flow characteristics of the solution for advantageous departure
from the printing nozzle and to improve the initial stability of printed constructs before the chemical cross-linking of alginate [29,51]. The polymer blend instantaneously gel once it is cooled below 10°C due to the thermoresponsive behavior of gelatin. Chemical cross-linking of alginate via CaCl$_2$ takes longer than the temperature-driven gelation mechanism of gelatin, requiring several minutes. This cross-linking should occur while the printed material is solidified in order to maintain structural integrity; otherwise, movement of the interface would result in unstable and inaccurate geometry [29].

Gelatin/alginate solutions containing sinus smooth muscle cells and aortic valve leaflet interstitial cells were bioprinted to form aortic valve conduits [51]. Increased alginate concentration caused poor mechanical integrity of the printed struts, while higher gelatin concentration resulted in high viscosity of bioink, which impaired deposition process. Both printed SMCs and aortic valve leaflet interstitial cells had cell viability more than 80% over 7 days. Cell spreading was found to increase over time that could be attributed to time-dependent dissociation of alginate by exchanges between Na$^+$ and Ca$^{2+}$. Tensile stress and modulus also decreased with culture time as a consequence of ion exchanges and the early release of gelatin [51]. Mechanical properties of printed constructs can be modified via changing the gelatin:alginate ratio, but the temperature has to be accurately controlled to avoid premature gelation of gelatin [29,51]. Also, there should be a balance between the amounts of gelatin and alginate accountable for immediate geometry stability and enhancing long-term stability.

### 3.2.3 Gelatin/Hyaluronan

Skardal et al. reported on the bioprinting of a polymer blend consisting of photocrosslinkable methacrylated hyaluronan (HA-MA) and gelatin ethanamine methacrylate (GE-MA) [26]. HA-MA is a promising material for bioprinting since its cross-linking degree can be easily controlled during the photopolymerization process. However, most cells cannot attach to HA-MA alone due to the lack of cell adhesive sites. Blending of HA-MA with a gelatin, which contains cell adhesion motifs, can help to enhance the cell viability. In this study, photocrosslinkable GE-MA was blended with HA-MA for bioprinting of cells [26]. Higher gelatin concentration gave rise to enhanced cell attachment compared to HA-MA only hydrogels. However, decrease in the modulus of the constructs was observed due to addition of GE-MA to HA-MA. The optimal composition was found to be 80% HA-MA 20% GE-MA, which provided adequate cell adhesion sites while maintaining the structural integrity of cell-laden bioprinted construct [26].

### 3.2.4 Hyaluronic Acid and Dextran

Hyaluronic acid is a linear polysaccharide component of ECM composed of $\beta$-1,4-linked $\alpha$-glucuronic acid ($\beta$-1,3) and N-acetyl-$\alpha$-glucosamine disaccharide units. Due to its viscoelasticity, excellent biocompatibility, and biodegradability, hyaluronic acid is one of the promising candidates for bioprinting. However, one drawback of bioprinting unmodified hyaluronic acid is the low stability of the construct due to its high water solubility [52]. Strategies aiming to reduce hydrophilicity of hyaluronic acid have been reported in literature, including derivatizing polysaccharide chains with hydrophobic moieties and/or cross-linkable chemical groups. Nevertheless, cell-laden constructs composed of only hyaluronic acid have not been successfully bioprinted [52].

Pescosolido et al. circumvented the instability of printed hyaluronic acid constructs by blending viscoelastic bioactive hyaluronic acid with photocrosslinkable dextran derivative, hydroxyethyl methacrylate derivatized dextran (dex-HEMA) [52]. Dextran is a bacteria-derived polysaccharide consisting of $\alpha$-1,6 linked $\alpha$-glucopyranose units with some $\alpha$-1,2-, $\alpha$-1,3-, and $\alpha$-1,4-linked side chains. Synthesis of dextran hydrogels can be achieved by radical polymerization of dextran derivatives with a reactive group [53,54]. Dex-HEMA can polymerize to form a gel in which hydrolytically sensitive esters are present in cross-links [53,54]. Since dex-HEMA is photocrosslinkable, a stable hydrogel can be formed after UV irradiation [52]. Mechanical properties of printed hyaluronan/dex-HEMA can be controlled by varying degrees of dextran derivative substitution as well as the concentration of dex-HEMA in the solution [52]. The solution of hyaluronan/dex-HEMA blend had a high viscosity at a low shear rate that is favorable for bioprinting. The rheological property of the polymer blend was mainly dominated by hyaluronan due to its high molecular weight (MW) and stiff polymer chains. Entangled hyaluronan chains efficiently dissipated deformation energy and consequently retarded network collapse. The printed constructs showed high cell viability of chondrocytes [52].

### 3.3 Natural Polymers for 3D Printing

Naturally derived bioinks are advantageous due to their intrinsic cytocompatible properties; however, limitations exist to tailor these polymers for biomedical applications. Some natural polymers, such as chitosan, silk fibroin, starch, and soy protein, have not been used for bioprinting. This is mainly attributed to printing and postprinting conditions, which are harmful to cells. Current and future research will attempt to circumvent these issues in order to better regenerate functional tissue. Their ability to form cell-encapsulated hydrogels with shear-thinning capabilities make them an attractive material for bioprinting bioinks. Improvements on mechanical integrity will be crucial for naturally derived bioink success, especially in larger constructs for organ replacement, although research seems to be on the right track. Some of these natural polymers that are used for 3D printing include chitosan, silk fibroin,
starch, and soy protein that will be highlighted in this section (Fig. 13.4).

3.3.1 Chitosan

Chitosan is a linear amino-polysaccharide composed of β(1–4) linked d-glucosamine residues and randomly located N-acetyl-glucosamine groups. It is a semicrystalline polymer that cannot dissolve in aqueous environments above neutral pH. In diluted acid, protonation of free amino acid groups generates a fully soluble molecule below pH 5. This solubility dependency allows chitosan to be used for bioprinting [55,56]. Viscous chitosan solution can be extruded and gelled by neutralization of acetic acid by sodium hydroxide (NaOH). To leach out residual NaOH, printed scaffolds are soaked in ethanol before being kept in deionized water. To remove excess water, the scaffolds are heated in the oven and then freeze-dried. The optimal range of NaOH concentrations was found to be between 0.75% and 1.5% v/v. Higher NaOH concentrations led to rapid gelation and consequently, little or no attachment between printed layers. On the other hand, low NaOH concentrations resulted in undesirable spreading of gel into the path of parallel struts, causing dragging of the overall constructs. Cells seeded on the scaffolds spread well and showed high viability. However, due to the harsh processing conditions, cells cannot be printed with the gel [55,56].

3.3.2 Silk Fibroin

Silk fibroin is a fibrous protein derived from the Bombyx mori silk worm. It is an amphiphilic block copolymer with a heavy chain composed of 12 repetitive domains predominated by the sequence G-X-G-X-G-X (G = glycine; X = alanine or serine). Eleven amorphous regions, consisting of more hydrophilic peptides, separate the dominating hydrophobic repetitive clusters [30,57]. Silk fibroin has excellent biocompatibility and robust mechanical properties. The molecular organization can transition between random coils to β-sheet through the addition of a poor solvent, such as methanol, which induces aggregation. This property allows silk fibroin to be used for 3D printing, that is, extruded struts crystallize when deposited into a methanol reservoir [30]. The system is optimized using 86% methanol to generate fibers that were elastic enough to maintain shape yet soft enough to stick to the layers underneath. Fibroin displayed shear-thinning behavior, that is, molecules aligned and the friction resistance between adjacent fibers decreased as shear rate increased. The optimal working concentration was found to be 29 wt%. At higher concentrations, chain mobility was suppressed and consequently hindered the transition into an aggregated structure [30].

However, printing silk-only solutions frequently led to clogged nozzles from shear-induced β-sheet crystallization. The unconstrained molecular mobility could be preserved by blending fibroin with gelatin [28]. These two polymers have opposite charges at physiological conditions (pH 7.2–7.4), yielding binding, which facilitated the smooth flow through nozzles [28]. Fibroin fractions in methanol transition from random coils to β-sheets, while gelatin fractions could undergo helix-random coil transition in the range of atmospheric and body temperatures. Consequently, structural integrity was maintained even at elevated temperatures. In addition, since fibroin does not have cell adhesion motifs, blending with gelation introduces integrin-recognizing RGD sequence that could enhance cell attachment and proliferation. Chondrocytes seeded on silk-only printed scaffolds were dedifferentiated whereas those seeded on fibroin/gelatin scaffolds exhibited round shapes indicating redifferentiation and maintenance of chondrocytic phenotype [28].

3.3.3 Starch

Starch is a polysaccharide produced by plants as energy storage. It is composed of two types of d-glucose: amylose and amylopectin. The ratio between the two glucose varies
according to the origin of the starch [27,58]. 3D printing of a blend of cornstarch, dextran, and gelatin powders in water was reported [27]. Using water as a binder is advantageous as problems of solvent residues and toxic fabrication environments can be eliminated. Starch granules do not dissolve in water but merely form a suspension. But, these starch granules swell and gelatinize to form a paste as the suspension is heated. Then, the amylose fraction separates from amyllopectin and forms a continuous phase surrounding swollen granules. As the starch cools down, the amylose phase separates leading to gel formation and rapid retrogradation. This results in a semicrystalline structure that is highly resistant to hydrolysis. Heat treatment at 100°C is necessary to dry and maintain structural integrity of printed constructs. This extreme temperature prevents the use of starch blend for bioprinting [27].

3.3.4 Soy Protein

Soy protein is plant-based with controllable properties through variable processing treatments. Its thermoplastic nature and biocompatibility allows the use of soy protein for 3D printing. Chien et al. reported on the 3D printing of this polymer for tissue engineering [59]. The printed constructs were treated with ethanol and freeze-dried to dehydrate proteins and increase structural stability. During this process, electrostatic interactions between proteins, PBS, and media ions promoted aggregates of protein. This is imperative for scaffold shape preservation as noncrosslinked constructs dissolve in water. Although soy protein can be printed at room temperature lacking organic solvents, which enables the incorporation of cells and growth factors, postprinting treatments create harsh conditions for cells, which hinder its use in bioprinting.

4 SYNTHETIC POLYMERS

While natural polymers provide a positive cell environment through mimicking native components of the ECM, synthetic polymers facilitate chemical manipulation of the structure to improve mechanical, biocompatible, and degradation properties. This chemical processing also enables researchers to generate cross-linkable structures for tissue engineering. In order to approach the biological recognition found in natural materials, these synthetic polymers can be equipped with molecular agents to enhance bioactivity as well as can be spatially deposited to stimulate cellular responses via mechanotransduction pathways [60]. These capabilities will be addressed in the following sections, along with relevant physical characteristics of the synthetic bioinks that have been developed thus far. Translatability of these materials used in 3D printing into bioprinting will also be considered as the utilization of toxic solvents or significant heat render them inhospitable to biological agents; however, concurrent printing of hydrogel bioinks is achievable. Some of these synthetic polymers that are used for bioprinting include PEG, PLGA, poly(ε-caprolactone), and PLLA that will be highlighted in this section (Fig. 13.5).

4.1 Poly(ethylene glycol)

Poly(ethylene glycol) is a hydrophilic, biocompatible, and FDA approved polymer that is extensively used in biomedical engineering through tissue engineering, drug delivery, and biosensors. PEG coating on polymeric nano- or microparticles results in significantly increased blood circulation time due to the nonfouling nature of PEG. In tissue engineering, the surface modifications of biomaterials surface...
via PEGylation facilitates easy control over cell-adhesion properties. PEG coating also results in biologically inactive surfaces that limit protein adhesion. Because of several astounding tunable properties, it has witnessed an unprecedented growth in tissue engineering applications in recent decades. At a structural level, the polyether is charge neutral; it has beneficial optical qualities, and it can be easily conjoined with multiple materials. Alterations to the MW and extent of cross-linking make it versatile for investigations of substrate elasticity and its effect on cell behavior, like in stem cell differentiation, which enables possible use in tissue engineering. Some of the resounding applications of PEG and its derivatives in bioprinting of scaffolds in recent times are discussed here.

PEG’s solubility in water makes it an attractive material for cell encapsulation, yet the polymer alone cannot form physical or chemical networks to result in a hydrogel. Thus, the polymer must be chemically modified prior to use as a bioink. Acrylation of this polymer has proven to be a key element toward achieving this goal of gel formation and, in this networked state, can stimulate cell differentiation. The general approach to accomplish this task is to cross-link the polymer chains via photoinitiator (PI)-induced polymerization under UV exposure. The use of UV light can be limited to preserve cell viability while still enabling sufficient free-radical generation for proper cross-linking. Prestwich et al. demonstrated the utility of acrylated PEG in the bioprinting of vascular grafts. In a typical experiment, they synthesized PEG-based multiarmed acrylated hydrogels of different chain lengths; TetraPEG-8 and TetraPEG-13 of 2 and 3.4 kDa MW, respectively, with acrylate group activated on both chain ends. The synthesized TetraPEGs were cross-linked with thiolated-hyaluronic acid and gelatin derivatives to form extrudable hydrogels (TetraPAC) for bioprinting applications. The extraordinary stiffness of these cross-linked hydrogel structures renders them suitable for printing tissue constructs especially vascular grafts as they retain physical integrity as well as fluidity during the high-pressure microcapillary pathway. The cellular viability of these hydrogels were examined using murine fibroblast (NIH3T3) cell lines before the cells were loaded at an optimal density in a calculated weight of TetraPAC13-cross-linked hydrogel, which could provide the maximum cell concentration with easy gelation and diffusion of nutrients and cellular metabolites in the constructs. To demonstrate the bioprinting applications, the researchers utilized a microcapillary tube printing process with an internal diameter of 500 μm. In the typical printing process, the prepared suspensions were filled into microcapillary to extrude microfilaments in a tubular orientation covered by agarose as a support mechanism to let the constructs remain intact.

There is an immense need for research in osteochondral repair and current implant therapies have not sufficiently addressed the exponential growth in the number of patients worldwide. D’Lima and coworkers exploited acrylated PEG for the quick and durable solution in this direction realizing the importance of UV-assisted cross-linked PEGDMA [18]. A typical cell-laden bioink based on PEGDMA was prepared using human articular chondrocytes stained with green and orange fluorescent dyes via UV exposure induced by photoinitiator Irgacure 2959. Here the simple methodology of PEG to generate terminal double bonds allowed rapid cross-linking in a solution conducive to cell viability. While these hydrogels would not be as stiff as the TetraPEG counterparts, they maintain the positive characteristics of the polymer, while also demonstrating favorable printing qualities. It is important to note that due to the lack of strong secondary forces present in the molecule, the $T_g$ of PEG is fairly low, even when cross-linked in a network. This results in greater chain mobility and thus a more rubbery material at body temperature, providing cells with a soft tissue mimicking material. The extent of cross-linking, therefore, will raise the $T_g$ and result in a stiffer bulk material [18]. Furthermore, a novel 3D thermal inkjet-based layer-by-layer deposition method was tested using prepared PEG bioink to repair osteochondral plugs serving as a 3D-biopaper. The obtained compressive modulus of bioink in the range of 400 kPa was found to be quite close to that measured for human articular cartilage. Further survival studies confirmed that cell-loaded hydrogels remained attached to both the adjacent cartilage and subchondral bone even after 6 weeks in culture and was supporting the larger proteoglycan production at their interface.

### 4.2 Poly(lactide-co-glycolide)

Despite the advent of strong cross-linking chemistry, most of the biopolymers applicable for bioprinting suffer from uncontrolled degradation and poor mechanical properties. PLGA being the copolymer of lactide and glycolide, obtained via ring-opening polymerization, has fetched wide attention as an alternative to overcome the listed drawbacks existing with other polymers. The popular condensation polymerization of d- and l-configurations can yield d,l-lactide, which is frequently used due to its improved toughness and easy manipulation of degradation rates. Some of the complex biostructures require immediate vasculature networks and involve cells like human umbilical vein endothelial cells (HUVEC), which need precise fluid flow control, invariable viscoelasticity, and fast solvent evaporation to be fabricated via a bioprinting method. PLGA emerges as a possible choice to fulfill these requirements. To accomplish this, the Ringeisen group conceived the idea of using PLGA as a stackable biopaper substrate to stack vascular cells to create high-resolution 3D tissue constructs via 2D biological laser printing technique. This technique has been previously used to print a variety of cell types including osteosarcoma cells [61], olfactory ensheathing cells [62], carcinoma cells [63], and bovine aortic endothelial cells [64], among several
others, and has been established as a hallmark in bioprinting techniques. The researchers in a typical experiment dissolved PLGA (MW 40–75 kDa) in chloroform and poured into PDMS molds with salt to fix it. After salt washing and solvent drying, the obtained scaffolds were filled with collagen and/or Matrigel, which provide the construct with biological sources. The HUVEC cells were then deposited in a stacked manner, which demonstrated the role of PLGA as a biopaper supporting the printing and transfer of 2D cell patterns and allowing the stacking of vascular cells to form 3D tissue structures. Their further experiments suggested that stacked HUVEC cells were more volatile to migrate or remained on the surface forming a network depending on whether the PLGA scaffold is either loaded with collagen or Matrigel, respectively. The employment of this material as a biopaper distinguishes this polymer from those previously presented as it is not strictly speaking a bioink due to its inability to incorporate cells directly into the solution. However, the rapid solvent evaporation and structural enhancements allow PLGA to enter the bioprinting realm as an additive biomaterial. Its ability to hydrolytically degrade ensures only temporary involvement in the tissue regeneration process and will allow cell mobility and remodeling to transpire.

4.3 Poly(ε-caprolactone)

PCL is a well-known biodegradable synthetic polyester, often used as plasticizer with several other polymers to design daily use household materials. It is a high MW semicrystalline polymer and imparts the additional elongation to the material by overcoming the brittle nature of conjugating copolymers like poly(lactic acid) and polyurethanes. PCL has been utilized in tissue engineering scaffolds for multiple applications due to its thermoplastic behavior, respectable mechanical strength, hydrolysis-induced biodegradation profile, and low melting point (approx. 60°C), which allows its easy processing. As a polyester, it exhibits nonenzymatic degradation and hydrolyzes to undergo sequential fragmentation in the primary and intermediate stages leading to bulk erosion eventually. Researchers have begun to introduce this polymer into 3D printing strategies to better spatially control PCL interconnectivity and porosity. However, the drawback with previously existing printing methods, like fused deposition modeling and precision extrusion deposition, are their dependency on extreme extrusion pressure on nozzle diameter to achieve maximum resolution. In that scenario, working with a viscous thermoplastic polymer like PCL would require very high pressure that sometimes goes beyond practical limits. Wei and Dong proposed the electrohydrodynamic jet (EHD-jet) technique where the polymer melt is subjected to an electrostatic field to form a conical structure releasing the fine jet with significantly better resolution than obtained with previous existing methods [65]. PCL being quite stable thermally, with better rheological properties, qualifies for fabrication in its melting phase using this technique. In a typical experiment PCL plates (MW 45 kDa) were used to form the conical jet under electrostatic field where the generated temperature gradient quickly solidifies the jetted PCL and form mechanically stable 3D constructs with resolution as good as 10 μm [65].

Again, similar to PLGA, the fabrication process limits the use of this polymer as a cell-laden bioink. The melting temperature of PCL (approx. 60°C) is too high to sustain cell viability; therefore, printing PCL using this method requires cell seeding after scaffold fabrication or via a separate bioink. Hence, PCL is not strictly a bioink that can encapsulate cells, but rather an additive network to provide hydrogel bioinks with a supportive structure. Because the PCL is utilized purely to reinforce the printed material rather than to mimic the ECM like in the traditional tissue-engineering paradigm, the construct is classified as a “scaffold-free” system. Scaffold-free systems aim to take advantage a cell’s inherent ability to organize themselves into complex structures via cell–cell interactions with minimal scaffold presence [66]. Researchers developed scaffold-free cell printing technology wherein the layer-by-layer deposition of cells can enable the construction of 3D organs. A novel modified printing technique with six dispensing heads is suggested using thermoplastic PCL and PLGA biomaterials to print the desired organ [41]. To demonstrate the system’s efficiency, heterogeneous cell lines, chondrocytes, and osteoblasts, were encapsulated within alginate solutions and infused within the PCL framework to construct 3D osteochondral plugs. The lamellae within the semicrystalline structure of this polyester provide the solidified form with mechanical properties relevant for soft tissue engineering. Amorphous regions also enable water penetration for hydrolytic cleavage of the polymer chain to result in suitable biodegradation as new tissue begins to inherit physiological stresses. It is noteworthy that the separately dispensed chondrocytes and osteoblasts remained viable for the next 7 days without significant fusion as confirmed by fluorescence microscopy, indicating a possible role of PCL frameworks in the regeneration of heterogeneous tissue constructs.

4.4 Poly(l-Lactic Acid)

PLLA is a well-known established aliphatic polymer. Its noteworthy mechanical properties (elastic modulus 1.5–2.7 GPa) and glass transition around 60°C, offers easy blending with many plasticizers to achieve desired rigidity. Furthermore, it exhibits process-related viscosity where low viscosity during extrusion allows adequate flow through an inkjet nozzle, while after printing, due to fast evaporation of the solvent, it becomes stiff and the printed structure stabilizes [67]. It is semicrystalline, biodegradable, biocompatible, and has found use in several medical applications like orthopedic implants, drug delivery systems, and biofabrication.
Therriault and coworkers selected PLLA for the fabrication of different microchannels using SC-DW (solvent-cast direct-write) method because of its resistance to thermal degradation at high temperatures and easy shape retention \([67,68]\). In the current work the researchers demonstrated the use of PLLA in fabricating square spiral, circular spiral, and microcup-shaped architectures following layer-by-layer extrusion technique where the extruded filament transitions from fluid state to viscous-solid state after extrusion, thus retaining the shape of the microarchitecture. It is imperative to mention that PLLA fluids exhibited shear-thinning behavior, which allows it to flow smoothly through the nozzle, and at the same time with faster jet speed, the solvent evaporation of extrudates could be enhanced. The researchers further proposed to extend the advanced 2D and 3D printing applications with similar thermoplastic polymers.

On a comparative note to PCL and PLGA, fabrication environments necessary for polymer printing once again inhibit simultaneous cellular interaction. PLLA has improved mechanical properties over PCL due to a shorter vinyl backbone and additional methyl pendant groups, decreasing the rotational mobility of the backbone. The \(T_g\) of \(\sim 60^\circ C\) is evidence of the increased backbone stiffness and overall chain motion restriction relative to PCL. Again, the semicrystalline morphology offers mechanical integrity while access for hydrolysis of the polyester, although hydrophobicity of the material may somewhat limit the presence of water, extend the lifetime of the material. Solidification kinetics and rheological properties of these inks alone have been investigated, although incorporation into bioprinted constructs has yet to be researched \([67]\).

5 SUMMARY

Due to biocompatibility concerns around fabrication methods involving synthetic polymers, cell-laden bioinks based purely from these biomaterials are uncommon. Synthetic polymers can include cell binding domains to enhance proliferation or be chemically modified for mechanical and degradation control. In order to take advantage of the mechanical strength found in many synthetics, further research is needed to enable cell manipulation with simultaneous printing through introducing natural materials or improving printer designs. 3D printing may provide some supplementary methods that bioprinting researchers could expand upon to realize the possibilities of synthetic bioinks. Furthermore, collaborative efforts are needed between printer design engineers and material scientists to generate synthetic polymer systems specifically for bioink applications.

6 POLYMER HYBRIDS

Similar to traditional tissue engineering, some bioprinting researchers have attempted to merge synthetic and natural polymer systems to more aptly control material properties. These hybrid polymer designs strive to incorporate the benefits of both types of polymers, for example, the tunability of synthetic materials with the biomimetic characteristics of natural polymers. Many of the synthetic polymers previously described, while useful for 3D printing, have limited use in bioprinting as the only biomaterial component; therefore, they can be an adjunct material when printed with another cell-laden ink. Due to the inherent complexity of native tissues, the usage of both types may be warranted for successful regeneration \([25]\). As previously mentioned, one group explored the usage of natural-based hyaluronan hydrogels cross-linked with tetrahedral PEG for the bioprinting of blood vessels \([35]\). Hyaluronic acid (HA) hydrogels alone demonstrate good biocompatibility and have been utilized for vessel repair \([69]\). PEG was introduced as a cross-linker due to its bioinert characteristic and its ability to covalently cross-link with natural polymers; in this case, thiolated HA once it has been processed to have terminal acrylate groups. Synthesized as a four-arm star polymer, this tetraPEG provided greater mechanical stiffness over HA cross-linked with PEG diacrylate (PEGDA) linkers. Interestingly, the chemical cross-linking between acrylate and thiol groups seemed to be hindered above a threshold value of cell density.

Copolymerization of synthetic and natural polymers has been utilized by tissue engineers to avoid the shortcomings of single polymer type systems. While the previously described synthetic polymers demonstrate acceptable mechanical strength and biocompatibility, they lack cell-recognizable binding sites to improve adhesion; however, hybridization of these polymers with natural polymers can better mimic the ECM, leading to superior cellular outcomes \([70]\). A fair amount of research has targeted natural/synthetic copolymers for traditional tissue engineering scaffolds; however, development of an extrusion system for these polymers has been limited \([71,72]\). In some cases, both polymer types are present in the deposition solution, but are not chemically conjugated to one another. For example, the presence of the natural polymer could increase bioink viscosity for better extrusion control during printing \([73]\).

Rather than a single bioink containing both synthetic and natural polymers, groups have developed hybrid systems that apply synthetic polymers as scaffolding for structure and shape, with the cell-laden naturally based bioink as a filler. This method has multiple advantages. First, synthetic thermoplastic polymers provide mechanical strength that typical hydrogel materials cannot provide \([25,74]\). Second, it does not limit the use of a single hydrogel, allowing for multiple hydrogels loaded with multiple cell types to be printed in a single construct \([25]\). PCL is popular as a supportive scaffolding due to its stiffness and degradation capabilities \([6,25]\). The thermoplastic polymer can be printed using an XYZ-controlled nozzle similar to applied
hydrogel to enable additional control over the final structure or through electrospinning; layers of randomly aligned PCL fibers can separate sections of hydrogels, which could allow variation of printed cell type and hydrogel material at different layers, as seen in Fig. 13.6. Additionally, lower viscosity hydrogels can be printed due to the mechanical strength provided by the PCL, enabling researchers to print with a greater amount of bioink materials.

7 EMERGING TRENDS AND FUTURE DIRECTIONS

While bioprinting itself has only just emerged as an exciting field of regenerative medicine research, recent trends have surfaced that attempt to propel these technologies into areas of even greater clinical relevance (Fig. 13.7). To overcome limitations associated with purely polymeric systems (e.g., insufficient mechanical strength and inefficient cellular stimulation), nanocomposites have been introduced as possible alternatives to improve upon these lacking characteristics [75–78]. Nanomaterials used in conjunction with polymer systems enable additional sites for cross-linking for mechanical stability or provide the cells with an alternate stimulus to motivate differentiation [79]. Multinozzle printing systems can also enhance mechanical integrity as well as inductivity through the inclusion of multiple materials acting in conjunction with one another. Both of these aspects are crucial for bioprinting design to more suitably mimic the native ECM.

An emerging trend in bioprinting is the development of multinozzle systems to print several bioinks within a single construct. Multiple bioinks allow investigators to integrate multiple cell types as well as polymer hydrogels to capture the complexity of the intended regenerated tissue [8,41,46,80,81]. Polymer bioinks could also include different growth factors that are spatially controlled in order to motivate stem cells into different lineages depending on their location within the printed material. While complex designs are a major advantage of these systems, they have the drawback of greater associated costs due to more advanced apparatuses [39]. As these multinozzle systems become more feasible for basic bioprinting research, polymer bioinks will be developed for specific printing applications as opposed to current bioinks, some of which were created with no considerations of bioprinting.

In order to better replicate this microenvironment, one group chemically functionalized 3D printed PLLA with multiwalled carbon nanotubes (MWCNTs), which have been shown to mimic collagen through similar size and shape, while also inducing stem cell differentiation into osteogenic and chondrogenic lineages. Polymer–nanocomposite interactions boosted mechanical strength of the modified scaffold, with a Young’s modulus similar to that of subchondral bone (30–50 MPa) [82]. By exposing the MWCNTs with poly-1-lysine after a H2 treatment, the MWCNTs became more hydrophilic and thus more biocompatible. Stem cells seeded directly onto the scaffold demonstrated increased proliferation due to the
bioinspired design. If instead of directly seeding cells onto the scaffold, they were encapsulated in a hydrogel bioink and printed layer-by-layer simultaneously with the functionalized PLLA-MWCNT scaffold, tissue formation could be improved further. Similarly, nanotitania has been dispersed in a printed PLGA scaffold to introduce surface roughness comparable to native bone [79]. Not only was osteoblast adhesion greater on well-dispersed scaffolds, tensile modulus also increased, which is vital for long-term construct success. Last, these nanoparticles shield the scaffold from the acidic degradation products of PLGA, reducing autocatalysis effects. Again, printing a polymer hydrogel bioink concurrently with the nanocomposite 3D scaffold could provide the correct microenvironment for tissue formation in vivo. These demonstrate the possible avenues of translation between the similar technologies of 3D printing and bioprinting.

In addition to polymer interactions with other nanomaterials, polymer bioinks can be integrated with growth factors to result in a bioactive scaffold. Due to the sensitivity to temperature, solvent, and conformation of these biological agents, they may be limited by fabrication method. Growth factors could be included directly in the polymer solution and then encapsulated during cross-linking [83]. Another mechanism to incorporate growth factors into a bioprinted material is through the use of degradable microspheres [84]. Charged domains on amino acids in growth factors and gelatin enable the formation of polyelectrolyte complexes within gelatin microspheres. Growth factors like BMP-2, transforming growth factor beta 1, or basic fibroblast growth factor can be contained within the microspheres, leading to a versatile carrier system for multiple regenerative applications. The microspheres are then dispersed within an alginate hydrogel containing a suspension of stem cells that can be printed with a defined architecture. Microspheres can be modified to vary release rates of the growth factors; however, size considerations are necessary to avoid clogging the nozzle during printing. The effect of extended BMP-2 exposure in scaffolds motivates osteogenic differentiation and therefore bone formation compared to immediate factor release. While the addition of the growth factor resulted in the desired cellular response, scaffold mechanical properties dissipated too quickly, which can be problematic if new stable tissue has yet to form [84].

To improve bioprinting materials and bring us closer toward functional tissue and organ replacement, several directions have been imagined for polymer bioinks. One of these proposed designs utilizes RGD peptides to augment cell adhesion within synthetic printed material, which could lead to better cellular fusion and enhanced function [14]. Bioinks composed of amphiphilic polymers that are capable of functionalization with bioactive agents could significantly improve upon cellular outcomes. These polymers would have the versatility to be printed as a gel or as microsphere cell encapsulation vehicles. “Active materials” are a novel type of polymer-based printing material that has recently been explored for device design, although they have not been employed for bioprinting usage [85]. As environmentally responsive materials, they demonstrate shape memory characteristics, which provide an extra
dimension of printing properties. While the true practicality of these “four-dimensional” materials for bioprinting would need to be demonstrated, one could envision a support system for a bioink, poly(N-isopropylacrylamide) for example, that transitions to a specific shape once implanted within the body or bioreactor to mold tissue formation. A more general outlook on future endeavors aims to determine optimal polymer “recipes” that provide a specific microenvironment to optimally stimulate encapsulated cells for a specific application. These formulations would be unique for each targeted physiological environment as well as a bioprinting method. Semi-IPN hydrogels have also been developed as bioinks to improve mechanical integrity to effectively mimic native tissue. The presence of one complete polymer network interlaced with non-cross-linked polymer strands distributes mechanical stress more effectively across the construct, while remaining highly swollen [86].

Last, one of the ultimate goals of bioprinting is to repair the body using in situ printing. A bioink capable of maintaining its complex structure and withstand biological and mechanical conditions within the body will be imperative. Additionally, nutrient transfer will be vital for continuous cell growth and function, particularly for larger organs that require greater depths of penetrating vasculature for nutrient and waste transfer.

8 CONCLUSIONS

The properties of bioink are very important in the bioprinting process to precisely design scaffolds for functional tissue engineering. Polymer bioinks should satisfy the following criteria: first, its rheological properties should allow smooth and uniform extrusion through fine nozzles without any choking or fractures. The printed polymer should shift from a fluid-like phase to solid-like phase soon once it exits the nozzle, maintaining the extruded form. In order to achieve this, the elastic modulus of the polymer bioink should be lower than the viscous modulus prior to printing. Second, once deposited the polymer should maintain its structural integrity. This means its elastic modulus should be greater than the viscous modulus. Polymer ink with shear thinning behavior also promotes this solidification process since the viscosity will increase significantly as shear disappears after extrusion. Third, it must provide cytocompatible environments for cells before, during, and after printing. Many bioinks fall short in regards to these guidelines. Some polymers require cytotoxic chemical cross-linkers or post-printing processing to enhance their mechanical integrity or remove coagulation solvents. These conditions (e.g., extreme temperature) could be too harsh for cell survival. As a result, not all polymers that are used for 3D printing, where cells are seeded after scaffold fabrication, can be used for bioprinting, where cells are printed together with the polymer bioink.

Polymer bioinks have great potential to revolutionize the field of tissue engineering. As a carrier of biological material that can be applied in complex structures at high resolution, bioinks provide bioprinting technologies with advantages over traditional scaffold fabrication. Specific regeneration applications require variable construct parameters and thus the consideration of bioink materials is vital to design success. Polymers can be functionalized to optimize design characteristics leading to enhanced regeneration outcomes. While many polymer bioinks have not yet reached their full potential, researchers have begun to pinpoint key properties necessary for cell viability and tissue formation. Similarly, new methods to print multiple polymer bioinks within a single design have improved outcomes. Further investigation into the interplay of polymer type and fabrication technique will help to formulate new polymer bioinks that can expedite the process from printing to implantation.

GLOSSARY

Biodegradation Chemical scission of covalent bonds via hydrolytic, enzymatic, or oxidative cleavage in a biological environment.
Bioink Printing material comprised of a nontoxic biomaterial utilized as a vehicle for cell deposition in a spatially controlled structure.
Biomimetic The engineering of biological structures based off of the structure or function of natural materials.
Extracellular matrix (ECM) A combination of structural components of tissues external to the cell.
Glass transition temperature \((T_g)\) Temperature range that results in increased segmental mobility of the polymer backbone.
Hydrogel A lightly chemically or physically cross-linked water swollen polymer network that retains its three-dimensional shape.
Polymer A material, both naturally and synthetically derived, from repeating unit structures, resulting in the formation of a macro-molecule.
Scaffold-free tissue engineering The arrangement of stem cells without structural components to guide growth or differentiation for tissue regeneration.
Thermoplastic Polymer type that softens into a more pliable phase upon heating, while becoming more rigid during cooling.
Viscoelasticity Type of deformation and recovery exhibiting the mechanical characteristics of viscous flow and elastic deformation.

ABBREVIATIONS

3D Three-dimensional
BMP-2 Bone morphogenic protein-2
ECM Extracellular matrix
GE Gelatin ethanolamine
HA Hyaluronan/hyaluronic acid
HEMA Hydroxyethyl methacrylate
MW Molecular weight
MWCNTs Multiwalled carbon nanotubes
PCL Poly(ε-caprolactone)
PEGDMA Poly(ethylene glycol) dimethacrylate
PI Photoinitiator
PLGA  Polylactic-co-glycolic acid
PLL A  Poly(l-lactic acid)
RGD  Arg-Gly-Asp
Semi-IPN  Semi-interpenetrating polymer network
TetraPEG  Four-arm PEG derivative
T<sub>g</sub>  Glass transition temperature
UCST  Upper critical solution temperature
VEGF  Vascular endothelial growth factor

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