Extrusion-Based Biofabrication in Tissue Engineering and Regenerative Medicine

Monika Hospodiuk, Kazim Kerim Moncal, Madhuri Dey, and Ibrahim T. Ozbolat

Contents

M. Hospodiuk (✉) · K. K. Moncal
The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA

Engineering Science and Mechanics Department, The Pennsylvania State University, University Park, PA, USA
e-mail: mxh96@psu.edu; kxm5653@psu.edu

M. Dey
The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA

Department of Chemistry, The Pennsylvania State University, University Park, PA, USA
e-mail: mxd5491@psu.edu

I. T. Ozbolat
The Huck Institutes of the Life Sciences, The Pennsylvania State University, University Park, PA, USA

Engineering Science and Mechanics Department, The Pennsylvania State University, University Park, PA, USA

Biomedical Engineering Department, The Pennsylvania State University, University Park, PA, USA

Materials Research Institute, The Pennsylvania State University, University Park, PA, USA
e-mail: ito1@psu.edu

A. Ovsianikov et al. (eds.), *3D Printing and Biofabrication*, Reference Series in Biomedical Engineering, https://doi.org/10.1007/978-3-319-45444-3_10

Abstract

Extrusion-based bioprinting is a powerful three-dimensional (3D) bioprinting technology that provides unique opportunities for use in organ fabrication. This technology has grown rapidly during the last decade. Extrusion-based bioprinting provides great versatility in printing various biological compounds or devices, including cells, tissues, organoids, and microfluidic devices that can be applied in basic research, pharmaceutics, drug testing, transplantation, and clinical uses. Extrusion-based bioprinting offers great flexibility in printing wide range of bioinks, including tissue spheroids, cell pellets, microcarriers, decellularized matrix components, and cell-laden hydrogels. Despite these assets, extrusion-based bioprinting has several limitations, such as inadequate control and resolution cell deposition, to create a complex tissue micro-microenvironment, shear stress-induced cell damage, and constraints associated with the current bioink materials.

1 Introduction

The extrusion process has been widely used in the fabrication of plastic and metal parts. The ease of complex structure formation and full process automation of extrusion printing drew the attention of tissue engineering and regenerative medicine scientists more than a decade ago (Vozzi et al. 2002; Yang et al. 2002; Pfister et al. 2004; Smith et al. 2004). Biocompatible hydrogels replaced plastics, and 3D printers were modified to print tissue scaffolds. Subsequent efforts were made to investigate the bioprintability of a wide range of soft materials blended with biological compounds. With the advent of live cell printing and the emergence of other bioprinting technologies, such as laser-based biofabrication (Odde and Renn 2000) and inkjet-based bioprinting (Pardo et al. 2003), the use of extrusion-based bioprinting had begun in earnest (Mironov et al. 2003). Extrusion-based bioprinting can be defined as the dispensing of a biological medium via an automated robotic system. During bioprinting, bioink is dispensed by a computer-controlled system, resulting in precise deposition of cells encapsulated in cylindrical filaments arranged in custom-shaped 3D structures.

Several researchers have demonstrated extrusion-based bioprinting of tissue substitutes (Ozbolat and Hospodiuk 2016). Various cell types have been loaded and deposited in a wide range of biocompatible hydrogels. Recently, artificial liver tissue constructs were engineered by encapsulation of hepatocytes within a gelatin methacrylamide (GelMA) hydrogel; cell viability in the construct was 97% after the bioprinting process (Billiet et al. 2014). In an adipose tissue engineering experiment, human adipose tissue-derived mesenchymal stem cells (hASCs) were loaded in a decellularized matrix. The bioink solution was printed in flexible dome shape in precisely defined patterns. Bioprinted cells showed significantly higher

adipogenic differentiation than hASCs cultured in nonprinted decellularized adipose tissue matrix (Pati et al. 2015a). Moreover, a new GelMA-based bioink containing gellan gum and mannose has been developed, which can be printed in a variety of 3D structures (pyramid, hemisphere, hollow cylinder) without compromising cell viability (Melchels et al. 2014). Kesti et al. showed 3D bioprinting of bovine chondrocytes into a complex 3D scaffold that was designed based on magnetic resonance imaging (MRI) scans (Kesti et al. 2015). Two types of hydrogels (bioink and support) were required in order to bioprint the nose- and ear-shaped patterns.

This chapter presents the principles of extrusion-based bioprinting including the extrusion mechanisms and the physical interactions that occur during extrusion. The bioink materials including hydrogels, decellularized matrix (dECM) components, cell aggregates, and microcarriers are presented along with their strengths and weaknesses. The limitations of extrusion-based bioprinting technology are discussed and future prospects are provided to the reader.

2 Extrusion-Based Bioprinting

2.1 Principles of Extrusion-Based Bioprinting

Extrusion-based bioprinting system relies on dispensing and positioning of a bioink solution through a nozzle on a computer-controlled motion stage. The blueprint design, which can be obtained from medical images, such as computed tomography (CT) and MRI, or freeform design models, is converted into a toolpath plan that is sent to the motion control system (Khoda et al. 2011). To bioprint the tissue construct automatically, an advanced control system that can simultaneously control both the motion of the bioprinter and the dispensing of the bioink solution is required. Here, we classify the dispensing mechanisms of extrusion-based bioprinting systems according to the means of extrusion.

2.1.1 Extrusion-Based Bioprinting Mechanisms

Extrusion-based bioprinting enables dispensing and writing of biomaterials such as cell-laden hydrogels (Khalil and Sun 2009; Chung et al. 2013; Murphy et al. 2013), cell aggregate-based biomaterials (Boland et al. 2003; Mironov et al. 2009; Jakab et al. 2010), and dECM (Pati et al. 2014) through an extrusion printhead driven by a computer-controlled dispensing system. A wide variety of cells can be combined with biomaterials, loaded into syringes and dispensed by pneumatic, mechanical, or solenoid-driven forces onto a printing platform, as shown in Fig. 1.

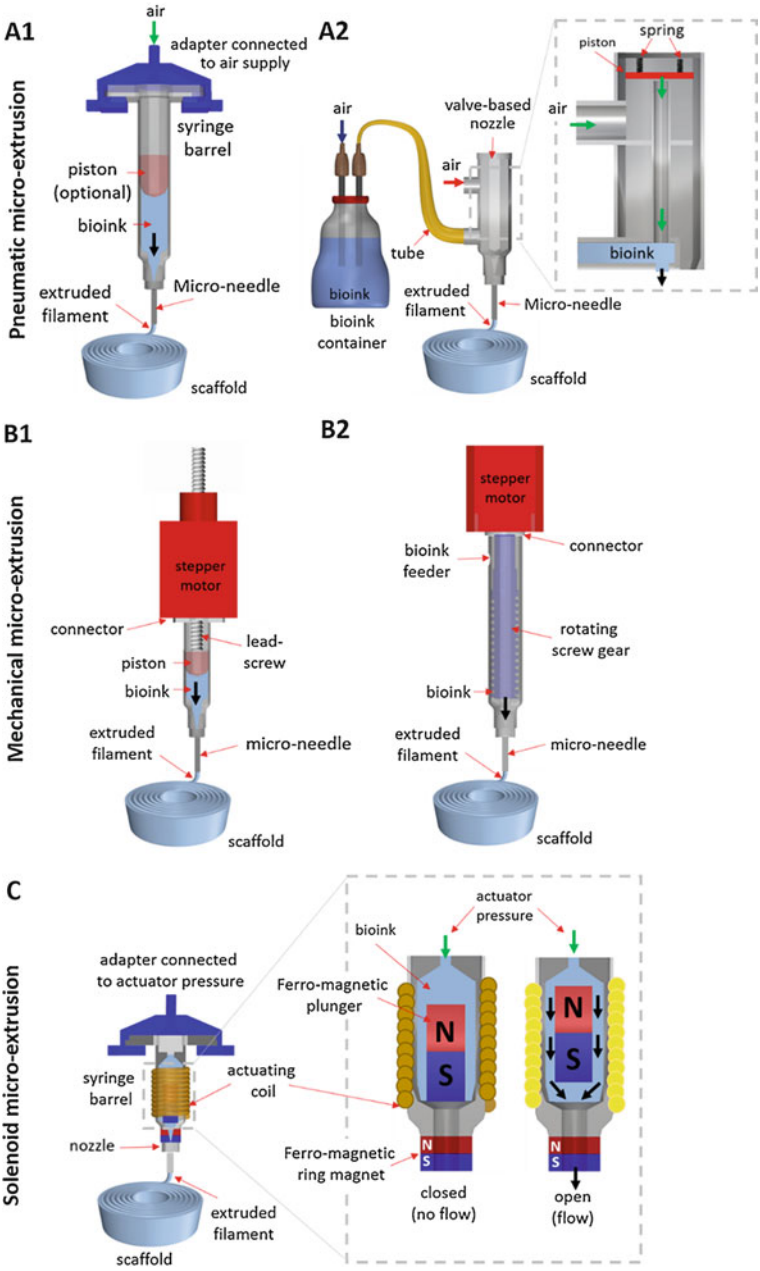

Fig. 1 Extrusion-based bioprinting mechanisms: (**A**) pneumatic micro-extrusion including (**A1**) valve-free and (**A2**) valve-based, (**B**) mechanical micro-extrusion including (**B1**) piston- or (**B2**) screw-driven and (**C**) solenoid micro-extrusion (Reproduced with permission from Ozbolat and Hospodiuk (2016))

Pneumatic-based extrusion system contains a pneumatic dispenser, which utilizes pressurized air with a valve-free (Fig. 1A1) or a valve-based (Fig. 1A2) configuration. The valve-free extrusion system has a syringe barrel that can be loaded with the bioink solution. An air pressure line is connected on top of the syringe barrel via an adapter to extrude the bioink solution through a micro-needle nozzle. When extrude pneumatically, the bioink solution must undergo certain shear stress. In that respect, hydrogels, which have shear-thinning properties, perform best in pneumatically driven extrusion-based bioprinting as they can maintain the filament shape upon extrusion. The valve-free extrusion system has been widely preferred due to its simplicity. However, for high-precision applications, a valve-based configuration is preferred since it is necessary to maintain a high degree of air pressure control and pulse frequency (Khalil et al. 2005). The valve-based system controls the air channel in the printhead via *on-off* valve switching that activates the air pressure externally when the valve is on and closes the channel when the valve is off. The bioink solution is dispensed from the bioink container through the printhead via air. In general, pneumatically driven systems have a high degree of precision and accuracy; a microdroplet size of 0.5 nL can be generated using a valve-based system. However, the cost of the system increases as the precision of the deposition volume increases. This approach has several disadvantages, as the pneumatic-based system requires sterilization of the air provided by a compressor. Thus, a filter in the airway must be used to minimize contamination of bioprinted structures. In order to extrude bioink solutions smoothly through the nozzle tip, the bioink solutions should be as homogenous as possible. If the bioink is in semisolid or solid form, it may require an additional liquid or gel medium to deliver the bioink solution successfully. Additionally, the bioink can easily attach on the wall of the nozzle. Due to their liquid nature, gel-based bioink solutions can easily transmit the extrusion force equally in all directions without entrapment inside the nozzle.

Mechanical micro-extrusion system is another mechanism which is preferred for the deposition of highly viscous materials such as synthetic and natural polymers. Mechanical extrusion can be designed in piston- (Fig. 1B1) or screw-driven (Fig. 1B2) configurations. The piston-driven mechanical extrusion system utilizes a piston connected to an electric motor. When the motor starts to rotate via electrical pulses, it converts the rotational motion to a linear motion. As a result, the piston advances in the barrel. The screw-driven configuration is useful for extrusion of bioink solutions with higher viscosity (Fielding et al. 2012). Furthermore, the screw-driven configuration can accommodate larger pressure drops along the nozzle. Mechanically driven systems are affordable, easy to program, portable, and do not need an air compressor unit and accessories. Moreover, sterilization of a mechanically driven system is simple as the mechanical dispenser head can be easily autoclaved. A mechanically driven system requires a tighter tolerance selection on the ram and the nozzle unit. An incorrect selection results in an unnecessary power draw on the motor, additional friction forces, leakage of bioink, or failure of the nozzle assembly due to overloading. Mechanically driven systems provide a better bioprinting ability for semisolid or solid bioink materials such as tissue strands.

Lastly, **solenoid micro-extrusion** (Fig. 1C) system mechanism operates via electric pulses. In order to open the valve, the magnetic pull force generated between a floating ferromagnetic plunger and a ferromagnetic ring magnet is canceled when the coil is actuated. Similarly, a piezoelectric-actuated system can be modified to dispense a sub-mL range volume of bioink solution (Bammesberger et al. 2013). Both systems are suitable for extrusion of low viscosity bioink solutions with an ionic- or UV-irradiation-based cross-linking mechanism. Additionally, the accuracy and reproducibility of the bioprinted constructs using this mechanism depend on several factors including the deflection time between actuation time (where the coil is energized) and the time when the valve opens. Highly viscous bioink solutions require a higher actuating pressure in order to extrude the bioink. Also, variations in the temperature and, hence, the viscosity, significantly, affect the valve opening time when the bioink has to be displaced in order to move the plunger. Solenoid-based micro-extrusion systems may not be ideal for thermally controlled nozzle configurations. In addition, re-calibration is required for the valve, especially if long dispensing tips are mounted. With that in mind, tolerance selection of the nozzle could be an important factor for the successful initialization of the bioprinting process.

Extrusion-based bioprinter systems provide a high degree of reproducibility between bioprinted scaffolds when appropriate bioink materials are utilized; in particular, hydrogels with suitable shear-thinning properties and rapid cross-linking capabilities can readily retain their printed shape. In addition, reproducibility of bioprinted constructs depends on several parameters such as dispensing tip diameter, viscosity of the bioink, bioprinter motion speed, extrusion force or pressure, and printing platform surface properties. The resolution of extrusion-based bioprinting is considerably lower than that of droplet- and laser-based systems. However, anatomically correct structures (Gou et al. 2014) and larger 3D constructs are rapidly generated. One of the most important aspects of nozzle selection is the friction coefficient on the wall of the nozzle tip; the friction coefficient mediates shear stress, which can be detrimental to live cells. Thus, a nozzle surface with a small friction coefficient that is easy to sterilize would be ideal for bioprinting cells (Bruzewicz et al. 2012).

2.1.2 Physical Interactions During Extrusion-Based Bioprinting

In order to dispense bioink solutions, the viscosity of the bioink should be able to quickly recover its original rheological state after extrusion. This is particularly important in order to maintain the shape of printed constructs. If the viscosity changes are reversible, the shear-thinning effect is observed (Jungst et al. 2016). Shear thinning is a time-independent property of a material's rheological behavior whereby the viscosity decreases when shear stress is applied and rapidly reverts to its original state as soon as the pressure is released. High-viscosity bioink will not flow through the printhead until pressure is exerted. Once shear stress is applied during extrusion, viscous bioink solutions behave like a liquid under pressure. Thus, bioink materials with shear-thinning properties are preferred as they can be held as a gel in a syringe barrel and extruded only when pressure is applied.

Extrusion-based bioprinting can change the viscosity of the bioink solution over time when mechanical or air pressure is applied. A viscosity change (particularly for non-Newtonian pseudoplastic hydrogels) that occurs in a time-dependent manner after the extrusion-induced disturbance is called thixotropy (Mewis and Wagner 2009). This behavior is similar to shear thinning except the reversion of the material to its original state takes place over time. In order to hasten and enhance reversibility, the bioink concentration can be increased using micro- or nanoparticles, which help decrease the relaxation behavior of the bioink during and after extrusion.

Lastly, in order to deposit the bioink successfully, surface properties of the printing stage should be optimized to stabilize the bioprinted filaments after extrusion. The surface adhesion and roughness of the printing platform is important in order for the filaments to stick and maintain their original shape. The printing surface should not warp or deform; bioprinting failure can result from attempting to print on an uneven surface.

2.2 Bioink

Extrusion-based bioprinting technology (see Fig. 2A) is a universal tool for deposition of a broad spectrum of bioink materials including, but not limited to, hydrogels, tissue spheroids, microcarriers, tissue strands and cell pellets, and dECM components. Material deposition is governed by the bioink type, a wide range of micronozzle diameters, and nozzle/tip designs.

2.2.1 Hydrogels: Characteristics and Limitations

A wide variety of hydrogels have been investigated for use in extrusion-based bioprinting. Depending on the cross-linking mechanism, hydrogels in extrusion-based bioprinting can be classified into three groups:

(i.) Physical (temperature (Duarte Campos et al. 2014) or light (Billiet et al. 2014))
(ii.) Enzymatic (Gregor and Hošek 2011)
(iii.) Chemical (pH (Smith et al. 2004) or ionic compound (Cohen et al. 2010))

Several review papers have been published on hydrogels used in tissue engineering (Drury and Mooney 2003); thus, this chapter focuses only on bioprintable hydrogels and their application and performance in extrusion-based bioprinting.

Alginate is an anionic polysaccharide which is present in brown seaweeds. It is made of copolymers of α-L-guluronic and β-D-mannuronic acid. Its biocompatibility, affordability, and fast gelation rate popularized this hydrogel in extrusion-based bioprinting (example shown in Fig. 3A) (Cohen et al. 2011; Jia et al. 2014; Wüst et al. 2014). Several extrusion-based bioprinting techniques have been investigated that exploit the rapid gelation property of alginate in ionic solutions of calcium (Ca^{2+}), such as calcium chloride, calcium carbonate, or calcium sulfate. These automated techniques include (i) bioplotting (Pfister et al. 2004), (ii) bioprinting with a secondary nozzle and with cross-linker deposition over hydrogel or a spraying system (Ahn et al.

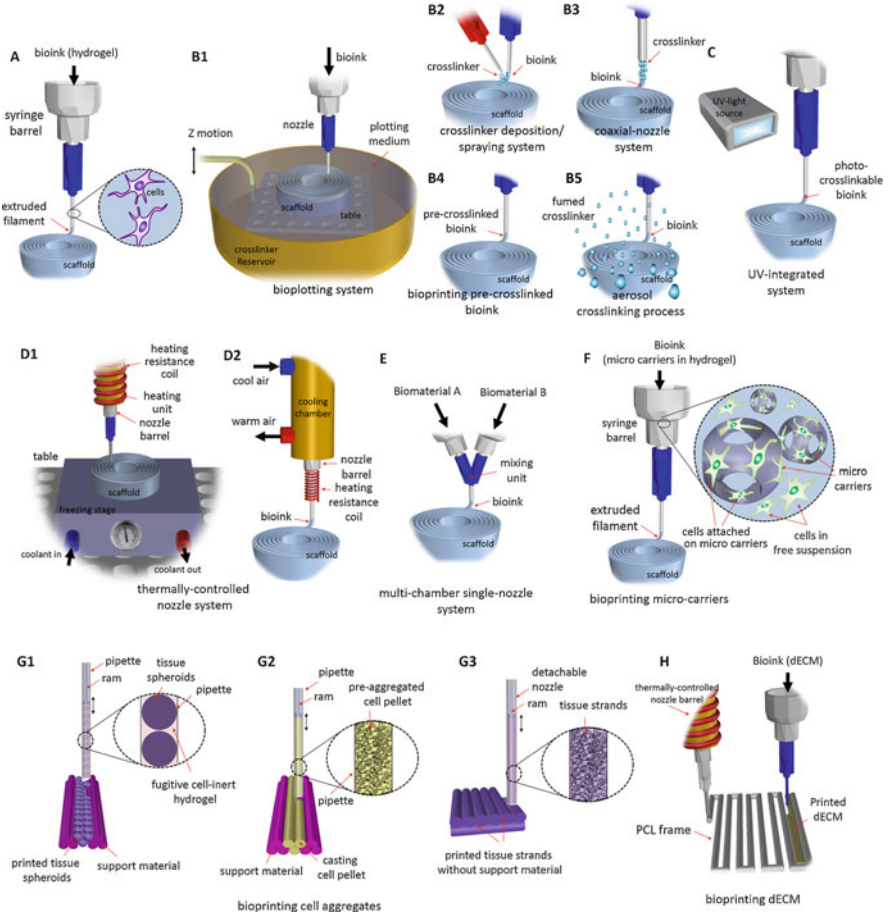

Fig. 2 Processes configurations for various bioink materials: (**A**) bioprinting cells in hydrogel-based bioink, (**B1**) bioplotting hydrogel bioink into a cross-linker reservoir, (**B2**) cross-linker deposition or spraying system, (**B3**) coaxial-nozzle system, (**B4**) bioprinting pre-cross-linked bioink, (**B5**) aerosol cross-linking system, (**C**) UV-integrated system, (**D1**) a heating unit-assisted barrel with cooling unit-assisted bioprinting stage, (**D2**) a cooling unit-assisted barrel with a heating unit-assisted nozzle tip, (**E**) multi-chamber single-nozzle system, (**F**) bioprinting microcarriers (preloaded with cells) that can be extruded in hydrogels as a delivery medium, (**G1**) extrusion of tissue spheroids in a fugitive cell-inert hydrogel into a support mold for fusion and maturation of spheroids, (**G2**) bioprinting pre-aggregated cell pellet into a support material that is inert to cell adhesion, (**G3**) bioprinting tissue strands directly without using delivery medium or support mold, and (**H**) bioprinting dECM within printed PCL frame to mechanically support gelation of dECM (Reproduced with permission from Ozbolat and Hospodiuk (2016))

2012a), (iii) bioprinting with coaxial nozzle-assisted technology (Ozbolat et al. 2014), (iv) bioprinting precross-linked hydrogel followed by full cross-linking after deposition (Chung et al. 2013), and (v) bioprinting hydrogel with an aerosol cross-linking system (Ahn et al. 2012b). Bioplotting is sometimes confused with "bioprinting," but

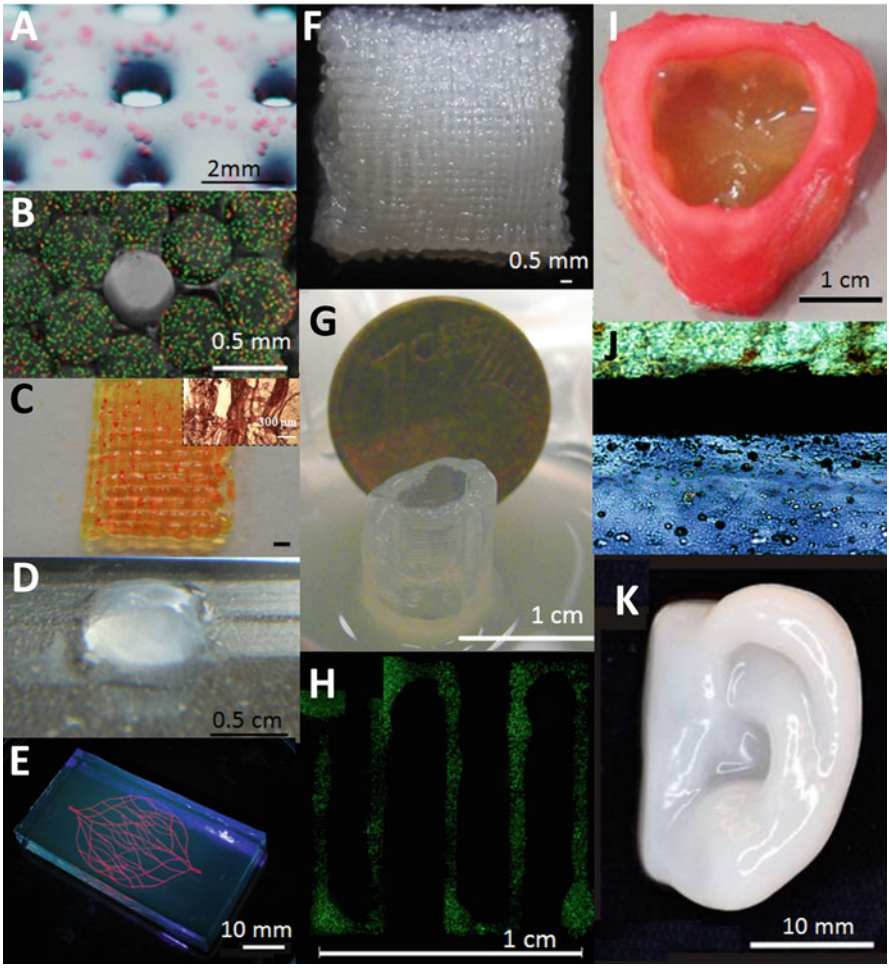

Fig. 3 Extrusion-based bioprinted constructs made of various hydrogels: (**A**) alginate scaffold with sustained release of proteins (*pink color*) (Reproduced with permission from Poldervaart et al. (2013)), (**B**) cell-laden GelMA constructs stained for live and dead cells (Reproduced with permission from Bertassoni et al. (2014), (**C**) 3D bioprinted Pluronic-collagen scaffolds stained with picrosirius red for visualization of collagen type I fibers, (**D**) 30 layers of fibrin forming a 3D scaffold (Reproduced with permission from Gregor and Hošek (2011)), (**E**) omnidirectional printing of Pluronic F-127 used for 3D microvascular network fabrication (Reproduced with permission from Wu et al. (2011b)), (**F**) chitosan scaffold with adipose stem cells extruded in a lattice pattern (Reproduced with permission from Ye et al. (2014)), (**G**) a cell-laden tubular agarose construct bioprinted into fluorocarbon (Reproduced with permission from Duarte Campos et al. (2013)), (**H**) live and dead staining of printed cells in Matrigel for a radioprotection study (Reproduced with permission from Snyder et al. (2011)), (**I**) PEG as aortic valve scaffold (Reproduced with permission from Hockaday et al. (2012)), (**J**) Alcian blue staining of a hyaluronic acid-based osteochondral-mimetic structure (PCL in black and collagen type I in turquoise color) (Reproduced with permission from Park et al. (2014)), (**K**) a nanocellulose scaffold in human ear shape for cartilage tissue engineering applications (Reproduced with permission from Markstedt et al. (2015))

they are not the same. In bioplotting (see Fig. 2B1), the hydrogel solution is deposited into a cross-linker pool, and the tissue construct stays within the pool until the process is completed. Therefore, the extrusion of bioink without a bioplotting pool is not considered "bioplotting." The next technique is shown in Fig. 2B2, where the cross-linker is deposited or sprayed onto the bioprinted hydrogel using a secondary nozzle that can rotate around the primary nozzle (Geng et al. 2005). In the third technique (see Fig. 2B3), alginate is bioprinted through an inner nozzle and the cross-linker is deposited through the outer nozzle. Using a similar approach but an opposite configuration, alginate can be extruded for fabricating core-shell fibers for controlled drug delivery (Zhang et al. 2012; Davoodi et al. 2014); bioprinting microfluidic channels for tissue engineering applications (Zhang et al. 2013), generally blood vessels (Zhang et al. 2015); and immobilizing cell pellets for tissue strand fabrication (Akkouch et al. 2015). In the fourth technique, as shown in Fig. 2B4, precross-linked alginate is bioprinted with a low cross-linker concentration, providing a bioink deposition that endures sufficient structural integrity of the tissue construct. This can be further enhanced by exposing the tissue construct to a second higher concentration cross-linker solution. This method deposits the bioink unevenly, resulting in discontinuities and proportional variation during the extrusion process. In the last approach, the cross-linker is fumed over the bioprinting stage using an ultrasonic humidifier (see Fig. 2B5). This process provides simultaneous cross-linking between layers and develops constructs that are well integrated mechanically and structurally. All these techniques have pros and cons; however, the systems showing promising results (well-integrated interlayers in 3D) are illustrated in Figs. 2B3–B4 (Ozbolat et al. 2014). Despite the advantages, alginate is hydrophilic; thus, cell surface receptors are unable to interact with an alginate matrix. Cells in alginate are immobilized; proliferation and intercellular interactions are limited. Additionally, mechanical properties of alginate are poor if low concentration is used; however, lower concentrations support greater cell viability and an improved proliferation rate. Furthermore, cells do not adhere easily to alginate surface unless modified with a hydrogel. Therefore, researchers attempted to modify alginate by addition of cell adhesion ligands as arginine–glycine–aspartic acid (RGD) peptides (Rowley et al. 1999), which significantly improved cell adhesion, spreading, and proliferation.

Gelatin is a denatured form of collagen (Gómez-Guillén et al. 2011). It is a thermally reversible hydrogel which is solid at low temperatures and characterized by instability under physiological conditions and low mechanical integrity. Gelatin is used in extrusion-based bioprinting with various chemical and physical modifications, i.e., metal ions or glutaraldehyde, to improve its bioprintability and stability (Wang et al. 2006; Xing et al. 2014). One of the common methods to stabilize gelatin at 37 °C is by chemical modification with methacrylamide (MA) side groups which enables photopolymerization of gelatin for cell encapsulation. To cross-link methacrylamide modified gelatin, a water-soluble photoinitiator is required (Van Den Bulcke et al. 2000). The resulting product, which is a GelMA, can be smoothly extruded with a pneumatic dispenser fitted with a UV light source (Billiet et al. 2014; Bertassoni et al. 2014). The reader is referred to Figs. 2C and 3B for a schematic of the process and an example of a bioprinted tissue construct, respectively. The

printability of GelMA relies on the following: the hydrogel concentration, cell density, and duration and intensity of UV curing. The duration of UV exposure affects cell viability, hydrogel density, and final stiffness of the printed tissue construct (Billiet et al. 2014; Bertassoni et al. 2014). In addition, chemically unmodified gelatin can used as a sacrificial material in fabrication of 3D printed constructs with open fluidic channels (Lee et al. 2010a). After printing, gelatin is liquefied by incubating the construct at 37 °C, creating perfusable channels within the construct. Fluidic networks within the construct enable the flow of nutrition, oxygen, and drugs throughout the construct.

Collagen type I has been broadly used in tissue engineering as a scaffold material (Achilli and Mantovani 2010). Collagen type I possesses the amino acid sequence RGD, which binds to cellular transmembrane receptors known collectively as integrins (Grzesik and Robey 1994). It mediates interactions between the cytoskeleton and ECM and serves as a signal conductor, activating various intracellular signaling pathways and cell functions. Collagen molecules dissolve in acids but cross-link when the pH, temperature, and ionic strength are adjusted close to physiological values. When collagen is neutralized to a pH between 7 and 7.4, collagen cross-links within 30–60 min at 37 °C (Park et al. 2014) making collagen an attractive candidate for in situ bioprinting. This type of bioprinting refers to the direct printing of tissue substitutes into a defect or lesion site in a clinical setting (Ozbolat 2015a). The collagen cross-linking mechanism is also compatible with thermally controlled extrusion-based bioprinting systems. The ideal time for extrusion is the instant polymerization of the collagen begins. Extruded collagen needs to be incubated at 37 °C until fully cross-linked in order to possess sufficient structural integrity, as shown in Fig. 3C. Bioprintability of collagen was first demonstrated by Smith et al. (Smith et al. 2004); collagen type I was blended with bovine aortic endothelial cells (BAECs) and bioprinted by a pneumatic-driven extrusion-based bioprinting system. The configuration presented in Fig. 2D2 is used to bioprint collagen; the bioink is maintained at approximately 4 °C then raised to physiologically relevant temperatures. After deposition, collagen can be fully cross-linked after about 30 min of incubation. Collagen type I has also been 3D printed with different cell types, as well as blended with natural or synthetic biomaterials for chapter ► "Fabrication and Printing of Multi-material Hydrogels" that enhance its bioprintability and mechanical properties (Rücker et al. 2006).

Fibrin has superior cell adhesion capabilities and is widely used in tissue engineering (Lee et al. 2010b; Yu et al. 2012; Ehsan et al. 2014). The cross-linking of fibrin is accomplished simply by mixing fibrinogen and thrombin solutions in an enzymatic reaction at room temperature or 37 °C. Based on the desired gel stiffness or cell adhesion properties, cross-linking conditions can be optimized by adjusting thrombin and fibrinogen concentrations. Despite its extensive use in biological applications, fibrin exhibits weak mechanical stiffness and rapid degradation. The rapid and irreversible cross-linking can cause difficulties during bioprinting, generating unstable structures after deposition (Murphy et al. 2013). There are several ways to deposit fibrin in an extrusion-based bioprinting system. One involves the separate deposition of the two fibrin components (fibrinogen and thrombin)

(Xu et al. 2006; Cui and Boland 2009). The second approach combines fibrinogen and thrombin on ice, inducing slow gelation; the material is then extruded using the arrangement illustrated in Fig. 2D2 (Fig. 3D shows an actual printed fibrin construct). The third method uses a multi-chamber single-nozzle approach, where thrombin and fibrinogen solution are blended into one solution at the end of the extrusion process, as shown in Fig. 2E (Gregor and Hošek 2011). In the fourth method, fibrinogen is blended with another hydrogel, bioprinted in a desired pattern, and then cross-linked with thrombin (Wei et al. 2007). Fibrin has great potential for in situ bioprinting applications as bioprinted fibrinogen can rapidly cross-link with naturally occurring thrombin in situ (Li et al. 2015a).

Pluronic® is a triblock polymer consisting of a poly(propylene oxide) core flanked by two poly(ethylene oxide) chains (PEO-PPO-PEO). Pluronic is temperature sensitive; the intermolecular assembly of PPO blocks leads to the development of micelle structures above critical temperature. For example, a 20% Pluronic F-127 solution is semi-sol at room temperature and gel above 20 °C. The sol–gel transformation can be modulated by adjusting the solution concentration (Skardal and Atala 2014). The temperature-dependent gelation of Pluronic F-127 makes it a superior bioink material for extrusion-based bioprinting technology (Wu et al. 2011b; Chang et al. 2011); however, it requires a thermally controlled extrusion mechanism. Therefore, a nozzle system with temperature control (shown in Fig. 2D2) is essential to solidify the bioink during the extrusion process. The bioink is loaded into the syringe barrel as a liquid and kept at low temperature in a cooling chamber. A heating unit surrounds the dispensing tip, enabling precise control of the extrusion temperature. This approach allows extrusion of Pluronic in solid form. Another option is a heated plate to prevent melting and deterioration of the structure and shape upon extrusion (see Fig. 4A). Spatially well-defined tissue constructs can be accurately bioprinted using Pluronic bioink (Smith et al. 2004). Although easily bioprinted, Pluronic has weak structural and mechanical properties and dissolves rapidly over time in aqueous solutions (Müller et al. 2015). Recent work, however, demonstrated that Pluronic could be chemically modified to improve its structural and biological properties (Melchels et al. 2016). Pluronic F-127 has been used as a sacrificial material, or fugitive ink (Wu et al. 2011b) as shown in Fig. 3E, or as a support material to create a vascular network (Homenick et al. 2011).

Chitosan is an antibacterial, antifungal, nontoxic, and biodegradable hydrogel produced by deacetylation of chitin; it has been used as wound dressing in regenerative medicine (Ong et al. 2008). It is widely used in the bone, skin, and cartilage tissue engineering, due to hyaluronic acid and glycosaminoglycan content that is similar to native tissue (Ma et al. 2003; Hong et al. 2007; Zhang et al. 2008; Hao et al. 2010). Chitosan is soluble in acid solutions and cross-linked by ionic and covalent agents; however, a water-soluble form at neutral pH ranges was found to facilitate gelation at about 40 °C (Rinaudo 2006). The disadvantage of chitosan in extrusion-based bioprinting is its slow gelation rate and weak mechanical properties. In order to maintain structural integrity of a printed shape for several hours, chitosan must be highly viscous (Hao et al. 2010; Ren et al. 2013). A multilayer printed

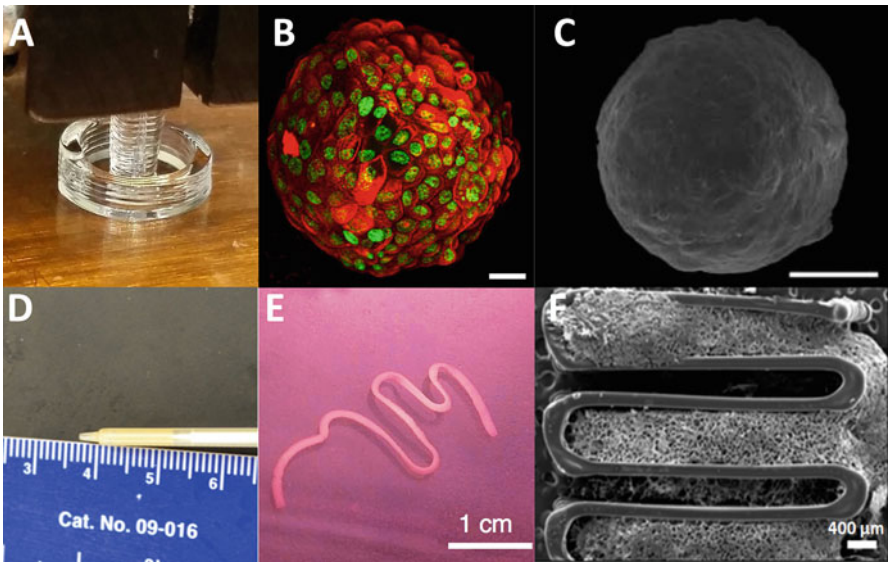

Fig. 4 Bioink types used in extrusion-based bioprinting: (**A**) Pluronic gel printed in concentric tubes, (**B**) polymer microcarriers preloaded with cells (scale bar 20 µm) (Reproduced with permission from Jakob et al. (2016)), (**C**) tissue spheroids made of cells and ECM (scale bar 100 µm) (Reproduced with permission from Norotte et al. (2009)), (**D**) cell pellet in nozzle tip, (**E**) tissue strands, and (**F**) hybrid printing of dECM with PCL framework (Reproduced with permission from Pati et al. (2014))

chitosan structure with embedded adipocytes is shown in Fig. 3F. To optimize strength and polymerization rate, chitosan can be blended with hydrogels with better mechanical properties. Chitosan has been used in extrusion-based bioprinting of various tissue constructs and devices, for example, microfluidic perfusable channels (Zhang et al. 2013), chondrogenic scaffolds encapsulated with adipose stem cells (Ye et al. 2014), and 3D printed scaffolds to study inflammatory responses (Almeida et al. 2014). The bioprinting mechanisms shown in Figs. 2B1–5 can be used in bioprinting of chitosan hydrogel.

Agarose is a polymer extracted from seaweed-based galactose with both thermoreversible and thermosensitive properties. Several types of agarose, with varying melting temperatures depending on the degree of hydroxyethylation, are available (Serwer et al. 1983). For extrusion-based bioprinting, the most suitable agarose is one with low-melting and low-gelling temperatures that is easy to liquefy and can solidify at 26–30 °C (Landers et al. 2002). The system configuration for agarose in extrusion-based bioprinting is presented in Fig. 2D1, where agarose is extruded in liquid state onto a cold stage resulting in rapid solidification. Recently, mesenchymal stem cells were encapsulated in agarose and then bioprinted in a tubular structure with fluorocarbon supporting the entire construct (see Fig. 3G) (Duarte Campos et al. 2013). The deposited cells are maintained in nearly 100% cell viability over 21 days. Agarose is also a suitable hydrogel for developing 3D cell

culture platforms; cells do not adhere to agarose but instead form large cell aggregates (Dean et al. 2007; Norotte et al. 2009; Mironov et al. 2011).

Matrigel, extracted from mouse Engelbreth–Holm–Swarm sarcoma cells and comprised primarily of laminin, collagen type IV, and enactin, forms a gelatinous protein mixture. The major advantage of Matrigel is the ability to support differentiation of many cell types and promote tissue outgrowth (Kleinman and Martin 2005). Another important characteristic of this hydrogel is its thermosensitive behavior (Wu and Ringeisen 2010). Matrigel is liquid at 4 °C but cross-links to form a gel at 24–37 °C. Complete gelation takes approximately 30 min and is a nonreversible process. Similar to collagen type I, in order to extrude Matrigel, the extrusion mechanism must possess a thermally controlled unit (as presented in Fig. 2D2) to hold the hydrogel at 4 °C. Extrusion-based bioprinting of Matrigel yielded high viability of human epithelial cells as shown in a live-dead staining image (see Fig. 3H) (Snyder et al. 2011). Moreover, bone marrow stromal cells bioprinted in Matrigel showed higher survival rates than cells encapsulated in alginate or agarose (Fedorovich et al. 2008). Also, multicellular constructs have been bioprinted and implanted in vivo for bone regeneration. Host tissue vascularization was demonstrated within 2 weeks after implantation (Fedorovich et al. 2011).

Poly(ethylene glycol) (PEG), as well as **poly(ethylene oxide)** (PEO), is widely used in non-pharmaceutical products and medical supplements (Giovagnoli et al. 2010; Mooney et al. 2011; Elbert 2012). The PEG-based hydrogels are biocompatible, are minimally immunogenic, and are approved by the Food and Drug Administration (FDA). One of the advantages of PEG-based hydrogels is their wide selection of cross-linking methods, using covalent, physical, or ionic agents (Veronese and Pasut 2005). Photopolymerization of PEG attracted major attention because of its tunable mechanical properties. A UV-integrated system can be used, as presented in Fig. 2C, to photocross-link polyethylene glycol diacrylate (PEG-DA) for quick 3D bioprinting of mechanically heterogeneous, complex, and clinically accurate scaffolds of aortic valve shapes (see Fig. 3I). Porcine aortic valve interstitial cells were seeded on the scaffold and cultured for up to 21 days (Hockaday et al. 2012). Unfortunately, cells seeded on the scaffold showed limited adhesion and spreading. Although PEG is a plausible option for cell encapsulation, it does not possess the cell adhesion motifs of bioink materials such as collagen. Hence, PEG-based materials need to be functionalized with the addition of cell binding sites growth factors during the bioprinting process to promote cell proliferation, migration, and regeneration of tissues (Zhang et al. 1998; Fedorovich et al. 2007).

Hyaluronic acid (HA) is an ubiquitous glycosaminoglycan present in most connective tissues (Oxlund and Andreassen 1980). During early embryogenesis, a high concentration of HA is present controlling a variety of cell functions and behavior, such as movement, angiogenesis, and proliferation. Hyaluronic acid is an attractive material for extrusion-based bioprinting due to its tunable physical and biological properties. Moreover, HA is the primary ECM component in cartilage tissue. A recent study revealed that 3D bioprinted chondrocyte-laden HA hydrogels (see Fig. 3J, in blue color) exhibited higher cell viability compared to cells loaded in collagen (Park et al. 2014). The disadvantages of HA are its poor mechanical

properties and rapid degradation rates (Jeon et al. 2007) that limit the use of HA in extrusion-based bioprinting; however, chemical modifications make it possible to control the degradation rate. One example is the functionalization of HA with UV-curable methacrylate (MA), where the duration of photopolymerization readily controls the degree of stiffness (Gerecht et al. 2007). Thus, the mechanism presented in Fig. 2C can be applied in the bioprinting of HA hydrogels functionalized with MA (Dana et al. 2004; Skardal et al. 2010b; Malda et al. 2013; Skardal and Atala 2014).

Methylcellulose (MC), a chemical compound obtained from cellulose as a semiflexible linear arrangement of polysaccharides, has the simplest chemical composition (Lott et al. 2013). Similar to gelatin and Pluronic, methylcellulose has thermosensitive and thermoreversible properties. The transition between sol and gel depends on the polymer concentration and molecular weight (Kobayashi et al. 1999). Methylcellulose in aqueous solution is used for cell culture purposes, as gelling occurs below 37 °C (Thirumala et al. 2013). Silanized hydroxypropyl methylcellulose hydrogel, a derivative of MC with pH-sensitive capabilities, has been patented and is used for 3D chondrogenic and osteogenic cultures (Trojani et al. 2005; Vinatier et al. 2005). Extrusion-based bioprinting of MC, as with other thermosensitive and thermoreversible hydrogels, requires an additional system such as a thermally controlled chamber (as presented in Fig. 3D2) and a heating stage. However, MC can be unstable exhibiting partial degradation with exposure to aqueous solutions such as cell culture media; hence, it is not suitable for long-term culturing of cells (Thirumala et al. 2013). Extrusion-based bioprinting of a bioactive glass with MC has shown good mechanical strength, which makes it an excellent candidate for use in bone regeneration (Wu et al. 2011a). Also, nanofibrillated cellulose has been blended with alginate, loaded with chondrocytes, and then bioprinted in the shape of a human ear, which demonstrates the ability of 3D bioprinting complex structures (see Fig. 3K) (Markstedt et al. 2015). Methylcellulose was also used as a scaffold matrix for corneal stromal cell spheroids fabricated under both rotary and static cell culture system, which were later used in a bioprinting procedure (Li et al. 2015b).

For simultaneous bioprinting of multiple hydrogels, a multi-chamber single-nozzle unit configuration has been developed, as shown in Fig. 2E. A major advantage of this approach is the ability to print one or more hydrogels to fabricate a heterogeneous construct with variations along the filament deposition direction (Ozbolat and Koc 2010). Biofabrication of hybrid porous tissue scaffolds have been demonstrated using the nozzle assembly, where various functional properties can be manipulated by modifying the nature and the concentration of the biomaterial used (Ozbolat and Koc 2011). The rate at which a bioink material is dispensed from the nozzle depends on the pressure applied using the pneumatic controller unit. An advanced design with a similar approach is the triple chamber (Mogas-Soldevila et al. 2014), where chitosan, sodium alginate, and chitin powder were blended in a static mixer nozzle, and scaffolds were fabricated using a six-axis robotic printer.

Synthetic as well as some naturally available hydrogels lack the proper constituents, such as native ECM proteins, for sustainable growth and proliferation of cells. Additionally, it is challenging to maintain a cell density similar to that of the native

tissue (Ozbolat 2015b). Hydrogels containing the RGD sequence, such as gelatin and collagen, or those with a fibrous microstructure, readily allow cell adhesion. In absence of such cell binding motifs, cells will not preferentially adhere and proliferate on a biomaterial surface. High concentrations of hydrogels result in a mechanically stronger construct but can be detrimental to cells. Hydrogels, such as Pluronic F-127, preserve the integrity when deposited in bulk but are unable to preserve mechanical and structural integrity when printed in filaments as they rapidly dissolve in culture media. In extrusion-based bioprinting, rheological properties of hydrogel play an important role, where bioink suspension must overcome surface tension-driven droplet formation and be extrudable in straight filaments form. The material can quickly spread over the printing stage if a very low concentration of the bioink is used as opposed to a highly viscous or concentrated bioink, which requires high pressure for continuous extrusion. However, increased pressure might induce cell lysis due to increased shear stress.

Another limitation in hydrogel-based bioinks is the degradation time of hydrogels and the production of potentially detrimental by-products. Generally, hydrogels in vitro degrade much slower than in vivo and the degradation behavior differs from hydrogel to hydrogel. Encapsulated cells often cannot deposit sufficient amounts of ECM and proliferate within a hydrogel to begin tissue reconstruction before construct degradation takes place in vivo. Degradation may also trigger a chronic inflammatory response in the host after implantation. Also, hydrogels should not produce toxic degradation by-products that are harmful to biologics. In summary, the hydrogel utilized in extrusion-based bioprinting should be compatible with the targeted tissue type and support cell growth and function until the tissue regeneration process is completed.

2.2.2 Other Bioink Types

Microcarriers are defined as small spherical carriers with a porous structure that provides an expanded surface area for cell attachment and growth (Fig. 4B). Commercially available microcarriers for bone and cartilage regeneration are made of dextran (Malda et al. 2003; Skardal et al. 2010a), polymers (Bayram et al. 2005; Curran et al. 2005), glass (Malda et al. 2003), gelatin (Liu et al. 2004), and collagen (Overstreet et al. 2003; Shikani et al. 2004). Cells proliferate more rapidly on microcarriers and exhibit improved interaction and aggregation inside microcarriers than the cells loaded in the hydrogel solution alone. Total surface area available for cell expansion is significantly higher than 2D culture (Levato et al. 2014). Extrusion-based bioprinting of microcarriers is similar to printing cell aggregates; however, due to polymer stiffness, printing may be difficult. Microcarriers are blended with a hydrogel solution, loaded into the barrel, and dispensed (see Fig. 2F). The major limitation of polymeric microcarriers is a prolonged degradation time and associated toxic by-products. Degradation of hydrogel-based microcarriers is dependent on concentration and material type. If microcarriers are made of hard polymers, they can clog the nozzle during extrusion process. Deposition of microcarriers is challenging, as precise delivery to ensure contact between microcarriers is essential to 3D development of the construct.

Cell aggregates are scaffold-free bioink materials used in self-assembly directed fabrication of tissues. The greatest advantage of this approach is the short fabrication required compared to scaffold-based approaches. Large numbers of cells are initially seeded resulting in rapid deposition of native ECM biomolecules. Several approaches have been explored for fabrication of cell aggregates, particularly tissue spheroids, including hanging drop, pellet culture, nonadhesive micromold hydrogels, microfluidics by hydrodynamic cell trapping, liquid overlay, spinner flask, and rotating wall vessel techniques (Breslin and O'Driscoll 2013; Mehesz et al. 2011). It should be noted that not all of these techniques have been applied to bioprinting, but any of them can be considered as an alternative approach. Examples of homocellular, as shown in the Fig. 4C, and heterocellular tissue spheroids have been demonstrated in the literature (Hsiao et al. 2009; Torisawa et al. 2009). Despite the numerous advantages, cell aggregates present certain challenges when applied to extrusion-based bioprinting. One of the major difficulties is loading tissue spheroids into the nozzle, as shown in the Fig. 2G1 (Mironov et al. 2009). Moreover, aggregates need to be extruded in a delivery medium of either a fugitive ink or a thermosensitive hydrogel that does not allow cell adhesion. Also, cell aggregates fuse quickly which resulting in accumulation inside the nozzle tip thereby hindering printability. Post bioprinting and discontinuities in the printed tissue are possible if the tissue spheroids have not been deposited in close proximity. Also, permeability of tissue spheroids is lower than that of hydrogels, limiting the diffusion of oxygen and other nutrients. Hence, fabrication of spheroids over 400 μm in diameter induces hypoxia leading to cell death (Achilli et al. 2012). However, resilient cells (such as stromal cells) or cells that can tolerate hypoxia (such as chondrocytes) can overcome this issue. Neocapillarization inside the tissue spheroid is highly desirable for scale-up fabrication of tissues and organs. An example of naturally existing spheroids in the human body is lymph nodes. This tissue is composed of stromal cells such as fibroblasts, endothelial cells, and follicular dendritic cells, which provides physiological function in tissue ranging from a few millimeters to 1–2 cm long (Katakai et al. 2004). For neo vascularization of 3D printed and engineered tissues, cell aggregates need to be printed before becoming fully mature. Otherwise, mature tissue spheroids lose their potential to fuse and vascularize. Another type of dense cell suspension bioink material is a cell pellet, which can be molded into any shape, as shown in Figs. 2G2 and 4D (Ozbolat 2015b). The main limitation of the cell pellet is fabrication of large-scale tissues without using a temporary molding material. Therefore, tissue strands (Akkouch et al. 2015) (Figs. 2G3 and 4E) are considered as an alternative technique. These constructs are produced as elongated filaments using a custom-made nozzle apparatus. The labor-intensive method of preparing cell aggregates is eliminated in this technique. This technique also adds in the advantage of printing tissue strands with vasculature. Thus this method has great potential in generating larger-scale tissues and organ constructs (Yu et al. 2014; Yu and Ozbolat 2014).

Decellularized extracellular matrix has recently been developed as a hydrogel-free technique to transform natural cell matrix into a bioprintable material. This material has also been blended with other hydrogels for bioprinting purposes (Jang

et al. 2016). Native tissue ECM is decellularized and fragmented into small pieces for extrusion bioprinting (Pati et al. 2014). One of the major disadvantages of dECM is the extremely low yield of material after extraction. In addition, dECM loses its mechanical and structural integrity as well as some biochemical properties when it is fragmented. Therefore, polycaprolactone (PCL) can be used as a support structure for bioprinted dECM bioink (see Figs. 2H and 4F) (Pati et al. 2015b).

2.3 Limitations

Extrusion-based bioprinting is the most convenient technique for rapid fabrication of 3D cellular porous structures. This technology holds great promise for future organ fabrication and scale-up tissue engineering. However, it has several limitations that need to be overcome to enhance the potential of extrusion-based bioprinting systems in organ fabrication. The main disadvantage is the low printing resolution due to the large nozzle configurations of extrusion-based systems. The need for rapid gelation to encapsulate cells and form a stable 3D constructs in a shorter span of time limits the choice of bioink materials. The other impediment is the presence of shear stress caused by the extrusion process. Shear stress on the nozzle tip wall results in a lower cell viability in highly concentrated bioinks. Moreover, changes in the nozzle geometry, dispensing pressure, and bioink concentration could collectively induce cell death (Yu et al. 2013). In addition, accumulation of bioink material in the nozzle can result in nozzle clogging over time. Depending on the type of biologics being printed, a number of events might lead to clogging of the nozzle such as diffusion of the cross-linker solution into the nozzle, imprecise control of the temperature, early fusion of spheroids, coagulation of the bioparticles/microcarriers loaded in relatively small diameter nozzles, and heterogeneous bioink solutions.

As the field of bioprinting is rapidly expanding its range of applications, a wide variety of extrusion-based systems have been developed to increase its functionality including motion capability of the robotic arms with high degree of freedom, ease of operation, compact size, full-automation capability, and ease of sterilization (Dababneh and Ozbolat 2014). Despite its versatility and assets, extrusion-based bioprinting has some disadvantages when compared to other technologies. First, the resolution of the technology is quite limited; the minimum feature size is generally over 100 μm (Duan et al. 2013), which is considerably lower than the resolution of other bioprinting techniques (Dababneh and Ozbolat 2014). Therefore, cells cannot be precisely patterned and organized due to limited resolution. In addition, the bioink, in liquid or sol–gel state, requires shear-thinning ability to overcome surface tension-driven droplet formation to be extruded in the form of cylindrical filaments. Furthermore, gelation and solidification requirements for the materials limit the hydrogel options used in extrusion-based systems. Shear stress on the nozzle tip has a substantial effect on cell viability, especially if the bioink solution contains high cell density (Chang et al. 2008).

2.4 Future Perspectives

Extrusion-based bioprinting is a versatile technique owing to its flexibility in incorporating different bioink types, its ability to fabricate perfusable and porous tissue structures, and its capability to rapidly build large tissue constructs with enhanced mechanical and biological properties, which cannot be achieved using laser or droplet-based bioprinting. Although remarkable progress has been made in the field of bioprinting, more efficient and robust end products are needed in order to transition from basic research to pharmaceutics and clinics (Ozbolat 2015b).

In situ bioprinting is a very promising technology, which involves the bioprinting of porous tissue analogues into defects and lesion sites. These printed constructs can integrate with the endogenous tissue and producing a new vascularized tissue to complete the healing process. Only a few attempts have been made to in situ bioprint materials employing inkjet (Wang et al. 2009; Hussain et al. 2010) and laser-based bioprinting techniques (Lopes et al. 2014). Conversely, extrusion-based bioprinting offers wide flexibility in printing tissue analogues with controlled porous architecture. A pilot study was performed by Cohen et al., in which precross-linked sodium alginate was deposited into a defect on an ex vivo femur model (Cohen et al. 2010). This ex vivo defect model provided a translational step toward clinical in situ bioprinting, bringing the technology from bench to bedside. It is envisaged that in situ bioprinting can be effectively applied to deep dermal injuries, composite tissues and flaps, and calvarial or craniofacial defects during maxillofacial or brain surgeries.

Although considerable progress has been made in developing novel biomaterials, there is a great need for developing new bioinks with enhanced gelation capabilities, higher mechanical and structural integrity, and bioprintability, which will be well suited for extrusion-based bioprinting and adapted for soft tissues. This would usher in a new field of research under biomaterials and biofabrication, "bioprintable biomaterials." One of the major weaknesses in currently existing hydrogel-based bioinks is the lack of environment for promoting growth and differentiation of stem cells into multiple lineages (Ker et al. 2011). To mimic the native organizational structure of tissues and organs which consists of multiple cell types, it is essential to develop a bioink which would support a similar organization of the heterocellular tissue microstructure (Carrow and Gaharwar 2014). The structural and physical properties of the native tissue can be faithfully recapitulated by integrating chemical, mechanical, and physical stimuli. An ideal hydrogel material should be able to promote cell adhesion, proliferation, and differentiation toward multiple lineages, should possess appropriate mechanical integrity and structural stability to persist even after bioprinting, facilitate engraftment with the endogenous tissue without generating an immune response, possess shear-thinning properties to ease bioprinting and rapid gelation, and be abundant, affordable, and commercially available with appropriate regulatory guidelines for clinical use.

One of the exciting future directions in the field of bioprinting is the bioprinting of new types of organs. These organs can be tuned to perform specific functions such as augment the physiology of the human body beyond its normal capabilities or treat

diseases. The nature of such organs could be entirely biological in perspective or in the form of cyborg organs intertwining biology and electronics. A proof of concept cyborg organ has been recently demonstrated by Zhang et al. (2012) where bionic ears were printed using a hybrid approach. This technique involved bioprinting chondrocytes in alginate along with silver nanoparticles in the form of an inductive coil antenna. This cultured cyborg organ model was evaluated and was found to exhibit enhanced auditory sensing for radio frequency reception.

3 Conclusions

Over the past decade, extrusion-based bioprinting has proven to be a useful technique for tissue fabrication. There is a rapidly growing interest in extrusion-based bioprinting systems among researchers in the tissue engineering community due to the recent advances in bioink materials and new processes for vascularized tissue fabrication. Due to its greater flexibility in bioprinting of various bioink materials including hydrogels, dECM components, cell aggregates, and microcarriers, extrusion-based bioprinting has enabled fabrication of a wide array of tissue constructs, organ modules, and organ-on-a-chip devices. One of the major advances in this field relies on the development of superior bioink materials with fast gelation mechanisms, simplifying the process of extrusion and bioprinting. Overcoming the limitations of extrusion-based bioprinting including the improvement of bioprinting resolution, full automation of the technology, and development of novel bioink compositions would help translate this technique from bench to bedside.

Acknowledgments This work has been supported by National Science Foundation CMMI Awards 1349716 and 1462232. We thank Fisayo Olashore and Donna Sosnoski from the Pennsylvania State University for improving the quality of the paper. The authors are grateful to the support from the Engineering Science and Mechanics Department and the College of Engineering at the Penn State University. *The authors confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.*

References

Achilli M, Mantovani D (2010) Tailoring mechanical properties of collagen-based scaffolds for vascular tissue engineering: the effects of pH, temperature and ionic strength on gelation. Polymers (Basel) 2:664–680. https://doi.org/10.3390/polym2040664

Achilli T, Meyer J, Morgan JR (2012) Advances in the formation, use and understanding of multicellular spheroids. Expert Opin Biol Ther 12:1347–1360. https://doi.org/10.1517/14712598.2012.707181.Advances

Ahn S, Lee H, Bonassar LJ, Kim G (2012a) Cells (MC3T3-E1)-laden alginate scaffolds fabricated by a modified solid-freeform fabrication process supplemented with an aerosol spraying. Biomacromolecules 13:2997–3003. https://doi.org/10.1021/bm3011352

Ahn S, Lee H, Puetzer J et al (2012b) Fabrication of cell-laden three-dimensional alginate-scaffolds with an aerosol cross-linking process. J Mater Chem 22:18735. https://doi.org/10.1039/c2jm33749e

Akkouch A, Yu Y, Ozbolat IT (2015) Microfabrication of scaffold-free tissue strands for three-dimensional tissue engineering. Biofabrication 7:31002. https://doi.org/10.1088/1758-5090/7/3/031002

Almeida C, Serra T, Oliveira M et al (2014) Impact of 3-D printed PLA- and chitosan-based scaffolds on human monocyte/macrophage responses: unraveling the effect of 3-D structures on inflammation. Acta Biomater 10:613–622. https://doi.org/10.1016/j.actbio.2013.10.035

Bammesberger SB, Kartmann S, Tanguy L et al (2013) A low-cost, normally closed, solenoid valve for non-contact dispensing in the sub-µl range. Micromachines 4:9–21. 10.3390/mi4010009

Bayram Y, Deveci M, Imirzalioglu N et al (2005) The cell based dressing with living allogenic keratinocytes in the treatment of foot ulcers: a case study. Br J Plast Surg 58:988–996. https://doi.org/10.1016/j.bjps.2005.04.031

Bertassoni LE, Cardoso JC, Manoharan V et al (2014) Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. Biofabrication 6:024105. https://doi.org/10.1088/1758-5082/6/2/024105

Billiet T, Gevaert E, De Schryver T et al (2014) The 3D printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability. Biomaterials 35:49–62. https://doi.org/10.1016/j.biomaterials.2013.09.078

Boland T, Mironov V, Gutowska A et al (2003) Cell and organ printing 2: fusion of cell aggregates in three-dimensional gels. Anat Rec Part A Discov Mol Cell Evol Biol 272A:497–502. https://doi.org/10.1002/ar.a.10059

Breslin S, O'Driscoll L (2013) Three-dimensional cell culture: the missing link in drug discovery. Drug Discov Today 18:240–249. https://doi.org/10.1016/j.drudis.2012.10.003

Bruzewicz DA, Reches M, Whitesides GM (2012) Low-cost printing of PDMS barriers to define microchannels in paper. Changes 29:997–1003

Carrow JK, Gaharwar AK (2014) Bioinspired polymeric nanocomposites for regenerative medicine. Macromol Chem Phys. https://doi.org/10.1002/macp.201400427

Chang R, Nam J, Sun W (2008) Effects of dispensing pressure and nozzle diameter on cell survival from solid freeform fabrication-based direct cell writing. Tissue Eng Part A 14:41–48. https://doi.org/10.1089/ten.a.2007.0004

Chang CC, Boland ED, Williams SK, Hoying JB (2011) Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. J Biomed Mater Res B Appl Biomater 98:160–170. https://doi.org/10.1002/jbm.b.31831

Chung JHY, Naficy S, Yue Z et al (2013) Bio-ink properties and printability for extrusion printing living cells. Biomater Sci 1:763. https://doi.org/10.1039/c3bm00012e

Cohen DL, Lipton JI, Bonassar LJ, Lipson H (2010) Additive manufacturing for in situ repair of osteochondral defects. Biofabrication 2:035004. https://doi.org/10.1088/1758-5082/2/3/035004

Cohen J, Zaleski KL, Nourissat G et al (2011) Survival of porcine mesenchymal stem cells over the alginate recovered cellular method. J Biomed Mater Res A 96:93–99. https://doi.org/10.1002/jbm.a.32961

Cui X, Boland T (2009) Human microvasculature fabrication using thermal inkjet printing technology. Biomaterials 30:6221–6227. https://doi.org/10.1016/j.biomaterials.2009.07.056

Curran SJ, Chen R, Curran JM, Hunt J (2005) Expansion of human chondrocytes in an intermittent stirred flow bioreactor, using modified biodegradable microspheres. Tissue Eng 11:1312–1322. https://doi.org/10.1089/ten.2005.11.1312

Dababneh AB, Ozbolat IT (2014) Bioprinting technology: a current state-of-the-art review. J Manuf Sci Eng 136:061016. https://doi.org/10.1115/1.4028512

Dana N, Parker V, Meredith M et al (2004) Photocrosslinkable hyaluronan as a scaffold for articular cartilage repair. Ann Biomed Eng 32:391–397

Davoodi P, Feng F, Xu Q et al (2014) Coaxial electrohydrodynamic atomization: microparticles for drug delivery applications. J Control Release. https://doi.org/10.1016/j.jconrel.2014.12.004

Dean DM, Napolitano AP, Youssef J, Morgan JR (2007) Rods, tori, and honeycombs: the directed self-assembly of microtissues with prescribed microscale geometries. FASEB J 21:4005–4012. https://doi.org/10.1096/fj.07-8710com

Drury JL, Mooney DJ (2003) Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 24:4337–4351. https://doi.org/10.1016/S0142-9612(03)00340-5

Duan B, Hockaday L, Kang KH, Butcher JT (2013) 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. J Biomed Mater Res Part A 101 A:1255–1264. https://doi.org/10.1002/jbm.a.34420

Duarte Campos DF, Blaeser A, Weber M et al (2013) Three-dimensional printing of stem cell-laden hydrogels submerged in a hydrophobic high-density fluid. Biofabrication 5:015003. https://doi.org/10.1088/1758-5082/5/1/015003

Duarte Campos DF, Blaeser A, Korsten A et al (2014) The stiffness and structure of three-dimensional printed hydrogels direct the differentiation of mesenchymal stromal cells toward adipogenic and osteogenic lineages. Tissue Eng Part A:1–17. https://doi.org/10.1089/ten.TEA.2014.0231

Ehsan SM, Welch-Reardon KM, Waterman ML et al (2014) A three-dimensional in vitro model of tumor cell intravasation. Integr Biol (Camb) 6:603–610. https://doi.org/10.1039/c3ib40170g

Elbert DL (2012) Liquid-liquid two phase systems for the production of porous hydrogels and hydrogel microspheres for biomedical applications: a tutorial review. Acta Biomater 7:31–56. https://doi.org/10.1016/j.actbio.2010.07.028.Liquid-liquid

Fedorovich NE, Alblas J, de Wijn JR et al (2007) Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. Tissue Eng 13:1905–1925. https://doi.org/10.1089/ten.2006.0175

Fedorovich NE, De Wijn JR, Verbout AJ et al (2008) Three-dimensional fiber deposition of cell-laden, viable, patterned constructs for bone tissue printing. Tissue Eng Part A 14:127–133. https://doi.org/10.1089/ten.a.2007.0158

Fedorovich NE, Wijnberg HM, Dhert WJ, Alblas J (2011) Distinct tissue formation by heterogeneous printing of osteo- and endothelial progenitor cells. Tissue Eng Part A 17:2113–2121. https://doi.org/10.1089/ten.tea.2011.0019

Fielding G, Bandyopadhyay A, Bose S (2012) Effects of silica and zinc oxide doping on mechanical and biological properties of 3D printed tricalcium phosphate tissue engineering scaffolds. Dent Mater 28:113–122. https://doi.org/10.1016/j.dental.2011.09.010

Geng L, Feng W, Hutmacher DW et al (2005) Direct writing of chitosan scaffolds using a robotic system. Rapid Prototyp J 11:90–97. https://doi.org/10.1108/13552540510589458

Gerecht S, Burdick J, Ferreira LS et al (2007) Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. Proc Natl Acad Sci U S A 104:11298–11303. https://doi.org/10.1073/pnas.0703723104

Giovagnoli S, Tsai T, DeLuca PP (2010) Formulation and release behavior of doxycycline-alginate hydrogel microparticles embedded into pluronic F127 thermogels as a potential new vehicle for doxycycline intradermal sustained delivery. AAPS PharmSciTech 11:212–220. https://doi.org/10.1208/s12249-009-9361-8

Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP (2011) Functional and bioactive properties of collagen and gelatin from alternative sources: a review. Food Hydrocoll 25:1813–1827. https://doi.org/10.1016/j.foodhyd.2011.02.007

Gou M, Qu X, Zhu W et al (2014) Bio-inspired detoxification using 3D-printed hydrogel nanocomposites. Nat Commun 5:3774. https://doi.org/10.1038/ncomms4774

Gregor A, Hošek J (2011) 3D printing methods of biological scaffolds used in tissue engineering. Rom Rev Precis Mech Opt Mechatronics 3:143–148

Grzesik WJ, Robey PG (1994) Bone matrix RGD glycoproteins: immunolocalization bone cells in vitro. J Bone Miner Res 9:487–496

Hao T, Wen N, Cao JK et al (2010) The support of matrix accumulation and the promotion of sheep articular cartilage defects repair in vivo by chitosan hydrogels. Osteoarthr Cartil 18:257–265. https://doi.org/10.1016/j.joca.2009.08.007

Hockaday L, Kang KH, Colangelo NW et al (2012) Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. Biofabrication 4:035005. https://doi.org/10.1088/1758-5082/4/3/035005

Homenick CM, de Silveira G, Sheardown H, Adronov A (2011) Pluronics as crosslinking agents for collagen: novel amphiphilic hydrogels. Polym Int 60:458–465. https://doi.org/10.1002/pi.2969

Hong Y, Song H, Gong Y et al (2007) Covalently crosslinked chitosan hydrogel: properties of in vitro degradation and chondrocyte encapsulation. Acta Biomater 3:23–31. https://doi.org/10.1016/j.actbio.2006.06.007

Hsiao AY, Torisawa YS, Tung YC et al (2009) Microfluidic system for formation of PC-3 prostate cancer co-culture spheroids. Biomaterials 30:3020–3027. https://doi.org/10.1016/j.biomaterials.2009.02.047

Hussain I, Hussain SZ, Habib-ur-Rehman et al (2010) In situ growth of gold nanoparticles on latent fingerprints-from forensic applications to inkjet printed nanoparticle patterns. Nanoscale 2:2575–2578. https://doi.org/10.1039/c0nr00593b

Jakab K, Norotte C, Marga F et al (2010) Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication 2:22001

Jakob PH, Kehrer J, Flood P et al (2016) A 3-D cell culture system to study epithelia functions using microcarriers. Cytotechnology 1–13. https://doi.org/10.1007/s10616-015-9935-0

Jang J, Kim TG, Kim BS et al (2016) Tailoring mechanical properties of decellularized extracellular matrix bioink by vitamin B2-induced photo-crosslinking. Acta Biomater 33:88–95. https://doi.org/10.1016/j.actbio.2016.01.013

Jeon O, Song SJ, Lee K-J et al (2007) Mechanical properties and degradation behaviors of hyaluronic acid hydrogels cross-linked at various cross-linking densities. Carbohydr Polym 70:251–257. https://doi.org/10.1016/j.carbpol.2007.04.002

Jia J, Richards DJ, Pollard S et al (2014) Engineering alginate as bioink for bioprinting. Acta Biomater 10:4323–4331. https://doi.org/10.1016/j.actbio.2014.06.034

Jungst T, Smolan W, Schacht K et al (2016) Strategies and molecular design criteria for 3D printable hydrogels. Chem Rev 116:1496–1539. https://doi.org/10.1021/acs.chemrev.5b00303

Katakai T, Hara T, Lee JH et al (2004) A novel reticular stromal structure in lymph node cortex: an immuno-platform for interactions among dendritic cells, T cells and B cells. Int Immunol 16:1133–1142. https://doi.org/10.1093/intimm/dxh113

Ker EDF, Chu B, Phillippi J et al (2011) Engineering spatial control of multiple differentiation fates within a stem cell population. Biomaterials 32:3413–3422. https://doi.org/10.1016/j.biomaterials.2011.01.036

Kesti M, Eberhardt C, Pagliccia G et al (2015) Bioprinting complex cartilaginous structures with clinically compliant biomaterials. Adv Funct Mater 25:7406–7417. https://doi.org/10.1002/adfm.201503423

Khalil S, Sun W (2009) Bioprinting endothelial cells with alginate for 3D tissue constructs. J Biomech Eng 131:111002. https://doi.org/10.1115/1.3128729

Khalil S, Nam J, Sun W (2005) Multi-nozzle deposition for construction of 3-D biopolymer tissue scaffolds. Rapid Prototyp J 11:9–17

Khoda A, Ozbolat IT, Koc B (2011) Engineered tissue scaffolds with variational porous architecture. J Biomech Eng 133:011001. https://doi.org/10.1115/1.4002933

Kleinman HK, Martin GR (2005) Matrigel: basement membrane matrix with biological activity. Semin Cancer Biol 15:378–386. https://doi.org/10.1016/j.semcancer.2005.05.004

Kobayashi K, Huang C, Lodge TP (1999) Thermoreversible gelation of aqueous methylcellulose solutions. Macromolecules 32:7070–7077. https://doi.org/10.1021/ma990242n

Landers R, Hübner U, Schmelzeisen R, Mülhaupt R (2002) Rapid prototyping of scaffolds derived from thermoreversible hydrogels and tailored for applications in tissue engineering. Biomaterials 23:4437–4447. https://doi.org/10.1016/S0142-9612(02)00139-4

Lee W, Lee V, Polio S et al (2010a) On-demand three-dimensional freeform fabrication of multi-layered hydrogel scaffold with fluidic channels. Biotechnol Bioeng 105:1178–1186. https://doi.org/10.1002/bit.22613

Lee Y-B, Polio S, Lee W et al (2010b) Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture. Exp Neurol 223:645–652. https://doi.org/10.1016/j.expneurol.2010.02.014

Levato R, Visser J, Planell J et al (2014) Biofabrication of tissue constructs by 3D bioprinting of cell-laden microcarriers. Biofabrication 6:35020

Li C, Faulkner-Jones A, Dun AR et al (2015a) Rapid formation of a supramolecular polypeptide-DNA hydrogel for in situ three-dimensional multilayer bioprinting. Angew Chem Int Ed Engl 1–6. https://doi.org/10.1002/anie.201411383

Li H, Dai Y, Shu J, et al (2015b) Spheroid cultures promote the stemness of corneal stromal cells. Tissue Cell 47:39–48. https://doi.org/10.1016/j.tice.2014.10.008

Liu JY, Hafner J, Dragieva G et al (2004) Autologous cultured keratinocytes on porcine gelatin microbeads effectively heal chronic venous leg ulcers. Wound Repair Regen 12:148–156. https://doi.org/10.1111/j.1067-1927.2004.012205.x

Lopes AJ, Lee IH, Macdonald E et al (2014) Laser curing of silver-based conductive inks for in situ 3D structural electronics fabrication in stereolithography. J Mater Process Technol 214:1935–1945. https://doi.org/10.1016/j.jmatprotec.2014.04.009

Lott JR, McAllister JW, Arvidson S et al (2013) Fibrillar structure of methylcellulose hydrogels. Biomacromolecules 14:2484–2488. https://doi.org/10.1021/bm400694r

Ma L, Gao C, Mao Z et al (2003) Collagen/chitosan porous scaffolds with improved biostability for skin tissue engineering. Biomaterials 24:4833–4841. https://doi.org/10.1016/S0142-9612(03)00374-0

Malda J, van Blitterswijk C, Grojec M et al (2003) Expansion of bovine chondrocytes on microcarriers enhances redifferentiation. Tissue Eng 9:939–948. https://doi.org/10.1089/107632703322495583

Malda J, Visser J, Melchels FP et al (2013) 25th anniversary article: engineering hydrogels for biofabrication. Adv Mater 25:5011–5028. https://doi.org/10.1002/adma.201302042

Markstedt K, Mantas A, Tournier I et al (2015) 3D bioprinting human chondrocytes with nanocellulose-alginate bioink for cartilage tissue engineering applications. Biomacromolecules 150325142328001. https://doi.org/10.1021/acs.biomac.5b00188

Mehesz AN, Brown J, Hajdu Z et al (2011) Scalable robotic biofabrication of tissue spheroids. Biofabrication 3:25002

Melchels FPW, Dhert WJ, Hutmacher DW, Malda J (2014) Development and characterisation of a new bioink for additive tissue manufacturing. J Mater Chem B 2:2282. https://doi.org/10.1039/c3tb21280g

Melchels FPW, Blokzijl MM, Levato R et al (2016) Hydrogel-based reinforcement of 3D bioprinted constructs. Biofabrication 8:035004. https://doi.org/10.1088/1758-5090/8/3/035004

Mewis J, Wagner NJ (2009) Thixotropy. Adv Colloid Interface Sci 147–148:214–227. https://doi.org/10.1016/j.cis.2008.09.005

Mironov V, Boland T, Trusk T et al (2003) Organ printing: computer-aided jet-based 3D tissue engineering. Trends Biotechnol 21:157–161. https://doi.org/10.1016/S0167-7799(03)00033-7

Mironov V, Visconti RP, Kasyanov V et al (2009) Organ printing: tissue spheroids as building blocks. Biomaterials 30:2164–2174

Mironov V, Kasyanov V, Markwald RR (2011) Organ printing: from bioprinter to organ biofabrication line. Curr Opin Biotechnol 22:667–673. https://doi.org/10.1016/j.copbio.2011.02.006

Mogas-Soldevila L, Duro-Royo J, Oxman N (2014) Water-based robotic fabrication: large-scale additive manufacturing of functionally graded hydrogel composites via multichamber extrusion. 3D Print Addit Manuf 1:141–151. https://doi.org/10.1089/3dp.2014.0014

Mooney R, Haeger S, Lawal R et al (2011) Control of neural cell composition in poly(ethylene glycol) hydrogel culture with soluble factors. Tissue Eng Part A 17:2805–2815. https://doi.org/10.1089/ten.tea.2010.0654

Müller M, Becher J, Schnabelrauch M, Zenobi-Wong M (2015) Nanostructured pluronic hydrogels as bioinks for 3D bioprinting. Biofabrication 7:035006. https://doi.org/10.1088/1758-5090/7/3/035006

Murphy SV, Skardal A, Atala A (2013) Evaluation of hydrogels for bio-printing applications. J Biomed Mater Res Part A 101(A):272–284. https://doi.org/10.1002/jbm.a.34326

Norotte C, Marga FS, Niklason LE, Forgacs G (2009) Scaffold-free vascular tissue engineering using bioprinting. Biomaterials 30:5910–5917. https://doi.org/10.1016/j.biomaterials.2009.06.034

Odde DJ, Renn MJ (2000) Laser-guided direct writing of living cells. Biotechnol Bioeng 67:312

Ong SY, Wu J, Moochhala SM et al (2008) Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. Biomaterials 29:4323–4332. https://doi.org/10.1016/j.biomaterials.2008.07.034

Overstreet M, Sohrabi A, Polotsky A et al (2003) Collagen microcarrier spinner culture promotes osteoblast proliferation and synthesis of matrix proteins. In Vitro Cell Dev Biol Anim 39:228–234. https://doi.org/10.1290/1543-706X(2003)039

Oxlund H, Andreassen TT (1980) The roles of hyaluronic acid, collagen and elastin in the mechanical properties of connective tissues. J Anat 131:611–620

Ozbolat IT (2015a) Bioprinting scale-up tissue and organ constructs for transplantation. Trends Biotechnol 33:395–400. https://doi.org/10.1016/j.tibtech.2015.04.005

Ozbolat IT (2015b) Scaffold-based or scaffold-free bioprinting: competing or complementing approaches? J Nanotechnol Eng Med. https://doi.org/10.1115/1.4030414

Ozbolat IT, Hospodiuk M (2016) Current advances and future perspectives in extrusion-based bioprinting. Biomaterials 76:321–343. https://doi.org/10.1016/j.biomaterials.2015.10.076

Ozbolat IT, Koc B (2010) Modeling of spatially controlled biomolecules in three-dimensional porous alginate structures. J Med Devices 4:041003. https://doi.org/10.1115/1.4002612

Ozbolat IT, Koc B (2011) Multi-function based modeling of 3D heterogeneous wound scaffolds for improved wound healing. Comput Aided Des Applic 8:43–57. https://doi.org/10.3722/cadaps.2011.43-57

Ozbolat IT, Chen H, Yu Y (2014) Development of "multi-arm bioprinter" for hybrid biofabrication of tissue engineering constructs. Robot Comput Integr Manuf 30:295–304. https://doi.org/10.1016/j.rcim.2013.10.005

Pardo L, Wilson WC, Boland T (2003) Characterization of patterned self-assembled monolayers and protein arrays generated by the ink-jet method. Langimur 19:1462–1466

Park JY, Choi J-C, Shim J-H et al (2014) A comparative study on collagen type I and hyaluronic acid dependent cell behavior for osteochondral tissue bioprinting. Biofabrication 6:035004. https://doi.org/10.1088/1758-5082/6/3/035004

Pati F, Jang J, Ha D-H et al (2014) Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun 5:3935. https://doi.org/10.1038/ncomms4935

Pati F, Ha D, Jang J et al (2015a) Biomimetic 3D tissue printing for soft tissue regeneration. Biomaterials 62:164–175. https://doi.org/10.1016/j.biomaterials.2015.05.043

Pati F, Jang J, Lee JW, Cho DW (2015b) Extrusion bioprinting. Essentials of 3D biofabrication and translation. https://doi.org/10.1016/B978-0-12-800972-7.00007-4

Pfister A, Landers R, Laib A et al (2004) Biofunctional rapid prototyping for tissue-engineering applications: 3D bioplotting versus 3D printing. J Polym Sci Part A Polym Chem 42:624–638. https://doi.org/10.1002/pola.10807

Poldervaart MT, Wang H, van der Stok J et al (2013) Sustained release of BMP-2 in bioprinted alginate for osteogenicity in mice and rats. PLoS One 8:e72610. https://doi.org/10.1371/journal.pone.0072610

Ren X, Kim Y, Zhou J (2013) Design and fabrication of chitosan for application of artificial photosynthesis. J Mech Eng Automat 3:739–746

Rinaudo M (2006) Chitin and chitosan: properties and applications. Prog Polym Sci 31:603–632. https://doi.org/10.1016/j.progpolymsci.2006.06.001

Rowley JA, Madlambayan G, Mooney DJ (1999) Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials 20:45–53. https://doi.org/10.1016/S0142-9612(98)00107-0

Rücker M, Laschke MW, Junker D et al (2006) Angiogenic and inflammatory response to biodegradable scaffolds in dorsal skinfold chambers of mice. Biomaterials 27:5027–5038. https://doi.org/10.1016/j.biomaterials.2006.05.033

Serwer P, Allen JL, Hayes SJ (1983) Agarose gel electrophoresis of bacteriophages and related particles III. Dependence of gel sieving on the agarose preparation. Electrophoresis 4:232–236. https://doi.org/10.1002/elps.1150040309

Shikani AH, Fink DJ, Sohrabi A et al (2004) Propagation of human nasal chondrocytes in microcarrier spinner culture. Am J Rhinol 18:105–112

Skardal A, Atala A (2014) Biomaterials for integration with 3-D bioprinting. Ann Biomed Eng. https://doi.org/10.1007/s10439-014-1207-1

Skardal A, Sarker SF, Crabbé A et al (2010a) The generation of 3-D tissue models based on hyaluronan hydrogel-coated microcarriers within a rotating wall vessel bioreactor. Biomaterials 31:8426–8435. https://doi.org/10.1016/j.biomaterials.2010.07.047

Skardal A, Zhang J, McCoard L et al (2010b) Photocrosslinkable hyaluronan-gelatin hydrogels for two-step bioprinting. Tissue Eng Part A 16:2675–2685. https://doi.org/10.1089/ten.TEA.2009.0798

Smith CM, Stone A, Stewart RL et al (2004) Three-dimensional bioassembly tool for generating viable tissue-engineered constructs. Tissue Eng 10:1566–1576

Snyder JE, Hamid Q, Wang C et al (2011) Bioprinting cell-laden matrigel for radioprotection study of liver by pro-drug conversion in a dual-tissue microfluidic chip. Biofabrication 3:034112. https://doi.org/10.1088/1758-5082/3/3/034112

Thirumala S, Gimble J, Devireddy R (2013) Methylcellulose based thermally reversible hydrogel system for tissue engineering applications. Cells 2:460–475. https://doi.org/10.3390/cells2030460

Torisawa Y, Mosadegh B, Luker GD et al (2009) Microfluidic hydrodynamic cellular patterning for systematic formation of co-culture spheroids. Integr Biol (Camb) 1:649–654. https://doi.org/10.1039/b915965g

Trojani C, Weiss P, Michiels JF et al (2005) Three-dimensional culture and differentiation of human osteogenic cells in an injectable hydroxypropylmethylcellulose hydrogel. Biomaterials 26:5509–5517. https://doi.org/10.1016/j.biomaterials.2005.02.001

Van Den Bulcke I, Bogdanov B, De Rooze N et al (2000) Structural and rheological properties of methacrylamide modified gelatin hydrogels. Biomacromolecules 1:31–38. https://doi.org/10.1021/bm990017d

Veronese FM, Pasut G (2005) PEGylation, successful approach to drug delivery. Drug Discov Today 10:1451–1458. https://doi.org/10.1016/S1359-6446(05)03575-0

Vinatier C, Magne D, Weiss P et al (2005) A silanized hydroxypropyl methylcellulose hydrogel for the three-dimensional culture of chondrocytes. Biomaterials 26:6643–6651. https://doi.org/10.1016/j.biomaterials.2005.04.057

Vozzi G, Previti A, De Rossi D, Ahluwalia A (2002) Microsyringe-based deposition of two-dimensional and three-dimensional polymer scaffolds with a well-defined geometry for application to tissue engineering. Tissue Eng 8:1089–1098. https://doi.org/10.1089/107632702320934182

Wang X, Yan Y, Pan Y et al (2006) Generation of three-dimensional hepatocyte/gelatin structures with rapid prototyping system. Tissue Eng 12:83–90. https://doi.org/10.1089/ten.2006.12.ft-16

Wang Z, Wu W, Yang Q et al (2009) In-situ fabrication of flexible vertically integrated electronic circuits by inkjet printing. J Alloys Compd 486:706–710. https://doi.org/10.1016/j.jallcom.2009.07.044

Wei Xu, Xiaohong Wang, Yongnian Yan, et al (2007) Rapid prototyping three-dimensional cell/gelatin/fibrinogen constructs for medical regeneration. J Bioact Compat Polym 22:363–377. https://doi.org/10.1177/0883911507079451

Wu PK, Ringeisen BR (2010) Development of human umbilical vein endothelial cell (HUVEC) and human umbilical vein smooth muscle cell (HUVSMC) branch/stem structures on hydrogel

layers via biological laser printing (BioLP). Biofabrication 2:014111. https://doi.org/10.1088/1758-5082/2/1/014111

Wu C, Luo Y, Cuniberti G et al (2011a) Three-dimensional printing of hierarchical and tough mesoporous bioactive glass scaffolds with a controllable pore architecture, excellent mechanical strength and mineralization ability. Acta Biomater 7:2644–2650. https://doi.org/10.1016/j.actbio.2011.03.009

Wu W, DeConinck A, Lewis J (2011b) Omnidirectional printing of 3D microvascular networks. Adv Mater 23:H178–H183. https://doi.org/10.1002/adma.201004625

Wüst S, Müller R, Hofmann S (2014) 3D bioprinting of complex channels – effects of material, orientation, geometry and cell embedding. J Biomed Mater Res A. https://doi.org/10.1002/jbm.a.35393

Xing Q, Yates K, Vogt C et al (2014) Increasing mechanical strength of gelatin hydrogels by divalent metal ion removal. Sci Rep 4:4706. https://doi.org/10.1038/srep04706

Xu T, Gregory C, Molnar P et al (2006) Viability and electrophysiology of neural cell structures generated by the inkjet printing method. Biomaterials 27:3580–3588. https://doi.org/10.1016/j.biomaterials.2006.01.048

Yang S, Leong K-F, Du Z, Chua C-K (2002) The design of scaffolds for use in tissue engineering. Part II. Rapid prototyping techniques. Tissue Eng 8:1–11. https://doi.org/10.1089/107632702753503009

Ye K, Felimban R, Traianedes K et al (2014) Chondrogenesis of infrapatellar fat pad derived adipose stem cells in 3D printed chitosan scaffold. PLoS One. https://doi.org/10.1371/journal.pone.0099410

Yu Y, Ozbolat IT (2014) Tissue strands as "bioink" for scale-up organ printing. Conf Proc IEEE Eng Med Biol Soc 2014:1428–1431. https://doi.org/10.1109/EMBC.2014.6943868

Yu Y, Brouillette MJ, Seol D et al (2012) Functional full-thickness articular cartilage repair by rhSDF-1α loaded fibrin/HA hydrogel network via chondrogenic progenitor cells homing. Arthritis Rheum 1–30. https://doi.org/10.1002/art

Yu Y, Zhang Y, Martin J, Ozbolat IT (2013) Evaluation of cell viability and functionality in vessel-like bioprintable cell-laden tubular channels. J Biomech Eng 135:91011. https://doi.org/10.1115/1.4024575

Yu Y, Zhang Y, Ozbolat IT (2014) A hybrid bioprinting approach for scale-up tissue fabrication. J Manuf Sci Eng 136:061013. https://doi.org/10.1115/1.4028511

Zhang M, Desai T, Ferrari M (1998) Proteins and cells on PEG immobilized silicon surfaces. Biomaterials 19:953–960. https://doi.org/10.1016/S0142-9612(98)00026-X

Zhang Y, Venugopal JR, El-Turki A et al (2008) Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering. Biomaterials 29:4314–4322. https://doi.org/10.1016/j.biomaterials.2008.07.038

Zhang L, Huang J, Si T, Xu RX (2012) Coaxial electrospray of microparticles and nanoparticles for biomedical applications. Changes 29:997–1003

Zhang Y, Yu Y, Ozbolat IT (2013) Direct bioprinting of vessel-like tubular microfluidic channels. J Nanotechnol Eng Med 4:020902. https://doi.org/10.1115/1.4024398

Zhang Y, Yu Y, Akkouch A et al (2015) In vitro study of directly bioprinted perfusable vasculature conduits. Biomater Sci 3:134–143. https://doi.org/10.1039/C4BM00234B