

Concise Review: Bioprinting of Stem Cells for Transplantable Tissue Fabrication

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ABSTRACT

Bioprinting is a quickly progressing technology, which holds the potential to generate replacement tissues and organs. Stem cells offer several advantages over differentiated cells for use as starting materials, including the potential for autologous tissue and differentiation into multiple cell lines. The three most commonly used stem cells are embryonic, induced pluripotent, and adult stem cells. Cells are combined with various natural and synthetic materials to form bioinks, which are used to fabricate scaffold-based or scaffold-free constructs. Computer aided design technology is combined with various bioprinting modalities including droplet-, extrusion-, or laser-based bioprinting to create tissue constructs. Each bioink and modality has its own advantages and disadvantages. Various materials and techniques are combined to maximize the benefits. Researchers have been successful in bioprinting cartilage, bone, cardiac, nervous, liver, and vascular tissues. However, a major limitation to clinical translation is building large-scale vascularized constructs. Many challenges must be overcome before this technology is used routinely in a clinical setting. STEM CELLS TRANSLATIONAL MEDICINE 2017;6:1940–1948

SIGNIFICANCE STATEMENT

This concise review presents the evolving technology bioprinting and its major components with a particular focus on bioink materials consisting of biomaterials and living cells. Various stem cells, used as a bioink material for bioprinting processes, are discussed thoroughly and their use in fabrication of different tissue types are expounded.

ESSENTIALS OF BIOPRINTING

Bioprinting is the spatial patterning of living cells and other nonliving biologic materials using an additive manufacturing technique [1]. The materials are assembled using a computer-aided layer-by-layer deposition approach for fabrication of living tissue and organ analogs used in tissue engineering, regenerative medicine, pharmacokinetic, and other biological studies [2, 3]. This technique allows for precise control of the micro architecture and macro-architecture of tissues and organs, which is critical to the function of many biological tissues and organs. An assortment of bioinks are available including hydrogels, microcarriers, tissue spheroids, cell pellet, tissue strands, and decellularized matrix components. They are used with a variety of bioprinting processes, including droplet-, extrusion-, and laser-based bioprinting [4]. These diverse inks and techniques each have their own advantages and disadvantages [5], and allow for customization of a range of complex tissues including cartilage, bone, cardiac muscle, neural tissue, liver, and vasculature. Various bioinks are used to create

each of these tissue types. Bioink can be defined as the bioprintable material consisting of living cells, proteins and other biologics loaded into a matrix. They mimic the extracellular matrix (ECM) to support cells. An ideal bioink material should be biocompatible, bioprintable, affordable, cell-friendly, mechanically strong and structurally stable, and possesses the solidification ability by means of cross-linking (i.e., physical, enzymatic and ionic) or aggregation of cells.

CELL SOURCE

In order to bioprint tissues for transplantation, the cell component of the bioink should be autologous and patient specific. In addition, most tissues consist of multiple cell types, which all have various functions. Stem cells are thus a promising choice as they have the ability to differentiate into multiple cell types for fabrication of autologous tissues. Therefore, the use of stem cells is highly critical to process an appropriate bioink material. Bioprinting applications use multiple stem cell types including embryonic, induced pluripotent, and adult stem cells (Fig. 1).

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Embryonic Stem Cells

Embryonic stem cells (ESCs) are pluripotent stem cells isolated from the blastocyst stage of in vitro fertilized embryos [6]. The embryos are cultured on a feeder layer of irradiated mouse fibroblasts with growth factors. Newer methods have been developed to culture cells without the mouse feeder layer to decrease the risk of viral transfer [7]. Many ethical debates were sparked by the use of fertilized embryos and therefore other researchers starting using dead embryos [8] and single cell biopsy [9]. ESCs proliferating in culture for at least 6 months without differentiating, that appear genetically normal, are considered an ESC line and can be frozen and sent to other laboratories for use. They can then undergo directed differentiation into various cell types. However, ESC use in research in the U.S. is currently limited.

Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) are adult fibroblasts genetically reprogrammed to have an embryonic like state [7]. Mouse iPSCs were first described in 2006 and human iPSCs in 2007 [10]. Four transcription factors found to be important in ESCs were introduced into fibroblasts using viruses to generate iPSCs: *Oct3/4*, *Sox2*, *c-Myc*, and *Klf4*. *Oct3/4* expression levels determine the fate of the inner cell mass [11]. *Sox2* interacts with *Oct3/4* to control gene expression and is important in maintaining pluripotency [12]. *C-Myc* plays an important role in growth control and differentiation of cells [13] whereas *klf4* is important for stem cell renewal and maintenance of pluripotency [14]. iPSCs are considered pluripotent, but have a lower differentiation capacity than ESCs and also carry an increased risk of teratoma formation [15, 16]. Therefore, others have developed chemically-iPSCs in mice [17].

Adult Stem Cells

Bone Marrow Stem Cells. Bone marrow stem cells (BMSCs) are a type of adult stem cell found in bone marrow. Adult stem cells are multipotent and reside in an area called the “stem cell niche.” They remain quiescent until they are activated to maintain normal tissues or repair diseased and injured tissues. They typically exist in small quantities and have a limited capacity to divide in vitro. It is thought that they would not induce rejection after transplantation of differentiated cells, thereby eliminating the need for immunosuppressive drugs that have many harsh side effects. Bone marrow contains both hematopoietic stem cells and stromal stem cells. The stromal stem cells make up a small portion of the bone marrow and can generate many tissue types [7]. They require less in vitro manipulation than ESCs and iPSCs, and have a much lower rate of malignant transformation than iPSCs [18]. However, their differentiation potential decreases with increasing age [19, 20] and harvest of BMSCs requires a painful procedure.

Adipose Derived Stem Cells. Adipose derived stem cells (ADSCs) are another type of adult stem cell abundant in white adipose (fat) tissue. They were first isolated from lipoaspirates in 2001 [21], but can also be obtained via lipectomy. With over 235,237 liposuction procedures and 127,633 lipectomies performed in the U.S. in 2016 [22], they offer easy accessibility. The infrapatellar fat pad is an alternative source of ADSCs, which show optimal results for cartilage and bone engineering [23]. ADSCs are found in large numbers and have a longer lifespan than BMSCs [24]. They offer up to a fivefold increase in stem cell yield

compared to BMSCs [25]. They hold great promise for autologous tissue fabrication.

INK

Bioinks are often adapted from hydrogels and derived from natural polymers. They are sensitive to harsh processing environments and often have high water content. Therefore, they are printed at lower temperatures than traditional three-dimensional (3D) printing and mild crosslinking agents or conditions are used. These mild conditions also ensure cell viability. The components are also chosen for their structural, sacrificial, functional, or supportive characteristics. According to their base materials, bioinks can be classified into two major categories including scaffold-based and scaffold-free bioinks [26].

Scaffold-Based Bioinks

Scaffold-based bioinks consist of cells dispersed within hydrogels or decellularized matrix components (dECM), or seeded on microcarriers that help to create a conducive environment for cell proliferation as well as providing structural support. Both differentiated cells and stem cells can be used in bioinks. Ideal bioinks are accurate when printed, crosslinkable, maintain their properties after polymerization, biocompatible, and undergo controlled degradation and ECM production. The most common type of bioinks are hydrogels, which can be natural or synthetic. Natural hydrogels include collagen, fibrin, chitosan, and alginate. Synthetic polymers are artificial and therefore their properties are more controllable than natural polymers, but their long-term effects on cells are unknown. Common synthetic hydrogels include methacrylated gelatin, Pluronic, and polyethylene glycol (Table 1). Researchers often combine various hydrogels and other components to improve bioink characteristics. Decellularized matrix components are a newer bioink source obtained by chopping tissue into small fragments, lysing the cells, and extracting the remaining ECM. However, ECM loses its mechanical and structural integrity during processing; therefore, a supportive frame is required during bioprinting [2]. Microcarriers have recently been used in bioprinting to increase the cell density in bioinks. They are porous particles designed to promote cell attachment, survival, and expansion. They can easily be suspended in culture media due to their low density [41] and microcarrier/cell complexes can be embedded in hydrogels for use in bioprinting. However, limitations include nozzle clogging during bioprinting and possible toxic byproducts of degradation.

Scaffold-Free Bioinks

Cells in scaffold-free constructs are bioprinted without a supporting hydrogel and therefore cells are loaded in higher concentrations. Tissue strands, pellets, or spheroids can be created. They deposit their own ECM components, which provides support as well as facilitates cell to cell communication and maturation [2]. This approach is only compatible with extrusion-based bioprinting (EBB). Various methods have been used to facilitate the assembly of spheroids [42]. They are bioprinted in close proximity and allowed to fuse during maturation. Several challenges exist with spheroids including the requirement of a delivery medium (sacrificial ink) for extrusion, premature fusion causing nozzle clogging, and gaps between printed spheroids leading to leaky tissues. Cells can instead be bioprinted into an inert hydrogel mold as a pellet

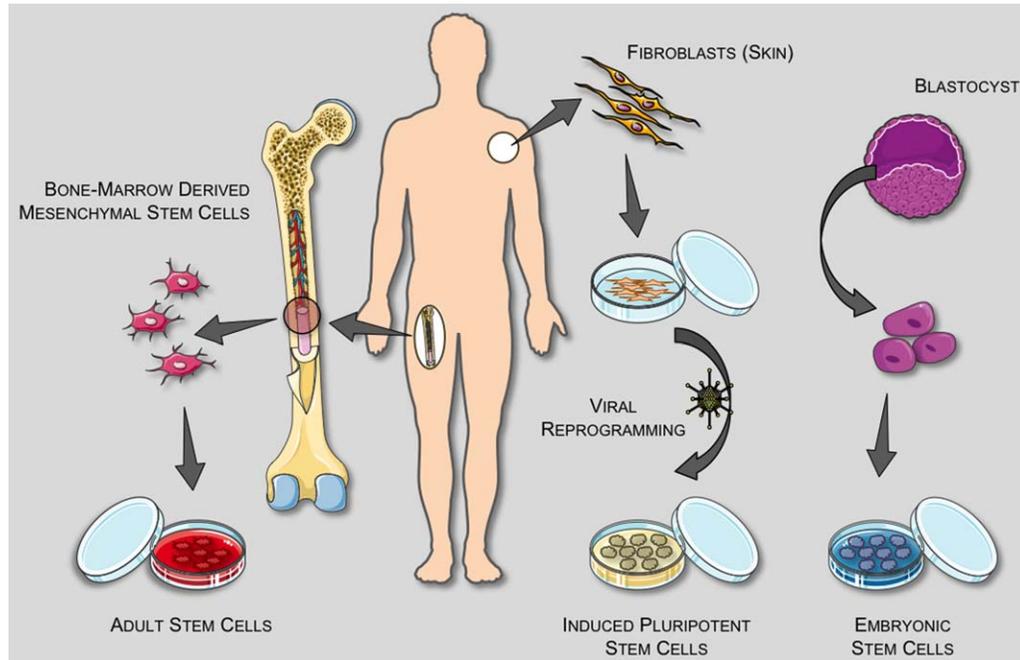


Figure 1. Stem cell sources. [Reproduced with permission from Christopherson, G. T. and Nesti, L. J. "Stem Cell Applications in Military Medicine" [Stem Cell Research & Therapy 2011;2:40]].

Table 1. Various natural and synthetic hydrogels with their type, advantages, and disadvantages

Hydrogel	Type	Advantages	Disadvantages	Cell type used	References
Agarose	Natural	High mechanical strength, low cost	Low cell adhesion	Human neural stem cells, porcine BMSCs	[27–29]
Alginate	Natural	Fast gelation, low cost, good stability	Poor cell attachment, easily clogs at high concentrations	Human neural stem cells, porcine BMSCs	[27, 28, 30]
Chitosan	Natural	Antibacterial & antifungal	Slow gelation, poor mechanical properties	Human neural stem cells, human ADSCs	[27, 29, 31]
Collagen I	Natural	Promotes cell attachment, good printing abilities, have RGD sequence ^a	Poor mechanical stability, slow gelation, easily clogs	Human amniotic fluid stem cells, human BMSCs	[29, 32]
Fibrin	Natural	Promotes angiogenesis, fast gelation	Poor mechanical stability, easily clogs	Human amniotic fluid stem cells, human BMSCs	[32, 33]
Gelatin	Natural	Reversible, promotes cell adhesion	Unstable/fragile, poor abilities without modification	Human cardiac progenitor cells	[34]
Hyaluronic acid (HA)	Natural	Promotes proliferation and angiogenesis, fast gelation	Rapid degradation, poor mechanical stability	Human cardiac progenitor cells, human BMSCs	[34, 35]
Matrigel	Natural	Promotes differentiation	Clogs easily, made from tumor cells	Human epithelial cells	[36]
Methacrylated gelatin/gelatin methacryloyl (GelMA)	Synthetic	Easily degradable, high mechanical strength	Slow gelation, requires ultraviolet (UV) light which causes cell damage	Porcine BMSCs, human BMSCs	[28, 37]
Pluronic	Synthetic	Reversible (good sacrificial ink)	Poor mechanical stability, rapid degradation, requires thermal control	Human endothelial cells, bovine chondrocytes	[39, 40]
Polyethylene glycol (PEG)	Synthetic	Good when combined with other components	Low cell proliferation & adhesion, poor mechanical strength, UV causes cell damage	Porcine BMSCs, human BMSCs	[28, 40]

^aRGD = tripeptide Arg-Gly-Asp sequence which mediates cell attachment.

Abbreviations: ADSCs, adipose derived stem cells; BMSCs, bone marrow stem cells.

and triggered to aggregate [43]. Final tissue size is limited by mold dimensions, which prevents easy human clinical translation. To overcome this, tissue strands were developed. Using a custom

nozzle, long strands can be printed without the use of a mold [44]. Scale-up tissues fabricated from cell aggregates will require vascularization as hypoxia occurs at diameters greater than

Table 2. Types of bioprinting modalities and their respective characteristics. Droplet-based bioprinting (DBB), extrusion-based bioprinting (EBB), and laser-based bioprinting (LBB). [data has been derived from [46]]

	DBB	EBB	LBB
Cost	Low ^a	Medium	High
Viscosity	<15 mPa/s	<6 x10 ⁷ mPa/s ^a	<300 mPa/s
Cell density	<10 ⁶ cells/ml	High, spheroids ^a	<10 ⁸ cells/ml
Print speed	Medium	Slow	Fast ^a
Resolution	50–100 μm	100 μm	20 μm ^a
Common bioinks	Agarose, alginate, collagen, fibrin, methacrylated gelatin, polyethylene glycol	Alginate, hyaluronic acid, polyethylene glycol, agarose, collagen, gelatin, pluronic, matrigel, fibrin ^a	Alginate, collagen, gelatin, matrigel
Cell viability	>85%	80%	95% ^a

^aDenotes best for that characteristic.

400 μm [45]. Due to these limitations, most researchers still use scaffold-based approaches [2].

BIOPRINTING PROCESSES

A medley of different bioprinting processes can be used to achieve the desired additive manufacturing goal and tissue fabrication. The three main modalities include droplet-, extrusion-, and laser-based bioprinting (LBB). These all have advantages and disadvantages, may be bioink specific, and are summarized in Table 2.

Droplet-Based Bioprinting

Droplet-based bioprinting (DBB) includes inkjet, acoustic-droplet-ejection, and microvalve bioprinting (Fig. 2A) [47]. Inkjet bioprinting was the first bioprinting technology developed and is the most commonly used type of droplet-based bioprinter. It is based on standard two-dimensional inkjet printing and a traditional printer can be modified [48]. The bioink is stored in a cartridge and manipulated to form droplets using gravity, atmospheric pressure, and fluid mechanics. Inkjet bioprinting can be further broken down into three types: continuous, drop-on-demand, and electrohydrodynamic [47]. Drop-on-demand requires high pressures to eject droplets through a nozzle, which can be harmful to cells whereas electrohydrodynamic jet bioprinters use an electric field to pull the bioink through limiting shear stress-induced cell damage.

Acoustic-droplet ejection bioprinting applies an acoustic field to eject droplets from a pool instead of a nozzle [49]. A piezoelectric substrate is used to generate acoustic waves and droplets are ejected when the force from the waves overcomes the surface tension. As a result, cells are not exposed to the stress of inkjet printing; however, the acoustic field can be easily disrupted leading to poor depositional control. In microvalve bioprinting, an electromechanical valve is used to generate droplets [50]. The bioink is housed within a pressurized fluid chamber and gated by a microvalve, then dispersed in a continuous manner or drop on demand depending on the pressure and gating time. Cell damage is limited, but larger droplets (50–300 μm) lead to a lower resolution. Highly complex constructs can be created with all types of droplet based bioprinters [51]. These bioprinters tend to be affordable and user friendly [47].

Extrusion-Based Bioprinting

EBB uses the shear thinning behavior of bioink materials and has progressed significantly in the past decade [2]. Bioink is deposited from a fluid dispensing system under control of a computer and

can be dispensed in cylindrical lines rather than droplets (Fig. 2B). Fluid deposition is driven by a pneumatic, mechanical, or solenoid system. Post-printing cell viability is usually around 80% but can be as high as 97% with optimization of process parameters [2]. Cell survival is decreased with increasing pressure, nozzle gauge, and shear stress. Computer aided design (CAD) software is easily incorporated and the continuous deposition improves structural integrity [52]. EBB also offers greater printing speed, facilitating scalability and clinical translation, and a larger variety of inks are able to be used. However, resolution is typically limited to 100 μm [53].

Laser-Based Bioprinting

LBB uses a donor layer comprised of a ribbon structure (Fig. 2C). A laser pulse creates a bubble at the interface and propels the bioink to form a droplet [54]. Mechanical stress is reduced because the technology is nozzle free and cells do not have direct contact with the printer (unlike the other bioprinting modalities), leading to high cell viability of >95% [51]. Additionally, highly viscous materials can be printed and resolution is the best of all methods. It is also highly precise and enables cells to be placed within 5 μm of the template [55]. Despite these benefits, the cellular effects of laser exposure are not known, lasers are expensive compared to the other systems, and the systems are large and complex.

BIOPRINTED TISSUES

Cartilage

Osteoarthritis affects 54.4 million people in the U.S. [56]. Bioprinting technologies are unique in that they enable the precise patterning of multiple cell types and materials to re-create the native structure of cartilage. Yu et al. fabricated scalable tissue strands using chondrocytes which were then bioprinted using a coaxial extrusion system to form larger tissues upon cell fusion, such as a cartilage patch [44]. Zhang et al. seeded MSCs in a 3D printed poly ε-caprolactone scaffold and placed them in meniscectomy defects in rabbits [57]. The scaffolds increased fibrocartilage tissue regeneration and mechanical strength, suggesting their potential as an alternative meniscal substitute. Nguyen et al. bioprinted iPSCs combined with irradiated chondrocytes and hyaline cartilage tissue which formed hyaline like cartilage with type II collagen expression (Fig. 3A) [58]. Recently, another group compared three different bioinks loaded with BMSCs (a

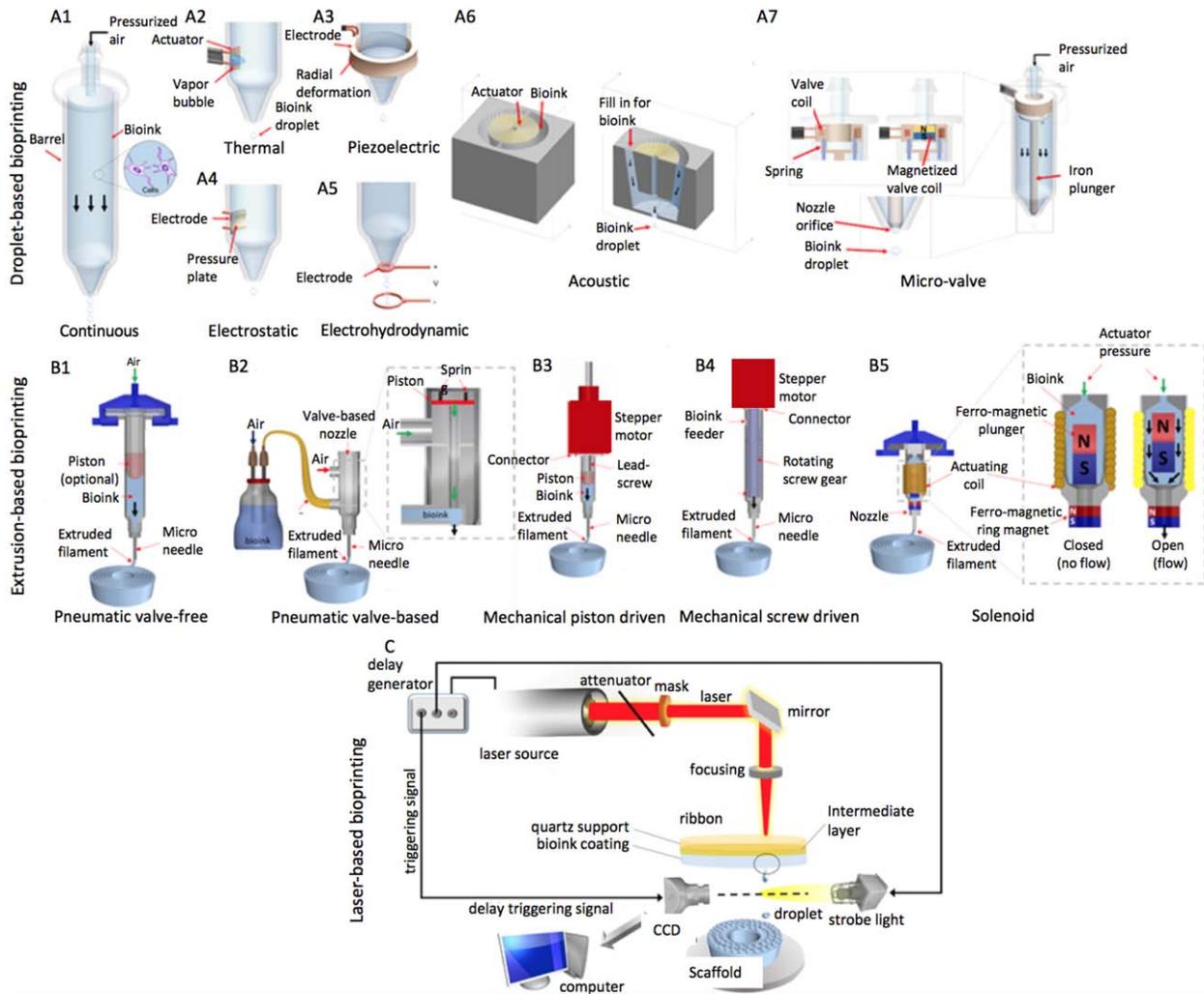


Figure 2. Bioprinting modalities. **(A):** Mechanisms of droplet-based bioprinting. Inkjet bioprinting techniques: **(A1):** continuous-ink-jetting relies on Rayleigh-Plateau instability, which breaks bioink jets into droplets; **(A2):** thermal drop-on-demand bioprinting uses a thermal actuator to locally heat bioink solutions to generate droplets; **(A3):** piezoelectric drop-on-demand bioprinting depends on radial deformation of a piezoelectric actuator to generate droplets; **(A4):** electrostatic bioprinting relies on deflection of pressure plate to generate droplets; **(A5):** electrohydrodynamic jetting uses an electric field, resulting from the electric potential difference between the printhead and the substrate, to pull a stream of bioink droplets through the printhead orifice. **(A6):** Acoustic-droplet ejection relies on a gentle acoustic field generated by an acoustic actuator to eject droplets from an open pool of bioink solution. **(A7):** Microvalve (solenoid) bioprinting operates with an electro-mechanical valve to dispense droplets. **(B):** Mechanisms of extrusion-based bioprinting. **(B1):** pneumatic microextrusion including **(B2)** valve-free and **(B3)** valve-based, **(B4)** mechanical microextrusion including **(B5)** piston- or **(B6)** screw-driven and **(B7)** solenoid microextrusion. **(C):** Schematics of laser printing setup based on laser-induced forward transfer: the upper donor slide is coated underneath with a thin laser energy absorbing layer and a layer of biological material to be transferred. The donor slide is placed above a second collector slide. Laser pulses are focused on the donor slide, evaporate the absorbing layer, and generate vapor pressure propelling the cell containing hydrogel toward the collector slide. Abbreviation: CCD, charge-coupled device.

GelMA, (b) GelMA + chondroitin sulfate aminoethyl methacrylate (CS-AEMA), and (c) GelMA + CS-AEMA + hyaluronic acid methacrylate [38]. The cells were then differentiated into chondrocytes post-printing. Enhanced viability and chondrogenic differentiation was seen as well as accuracy of the method, suggesting this as a model for engineering cartilage tissue.

Bone

Bony defects most commonly occur after trauma or tumor resection. Currently, hardware is placed or bone grafts are used to reconstruct defects, but many limitations exist. Pati et al. added ECM to their scaffolds to mimic the bony microenvironment and

showed upregulation of four osteoblastic genes as well as increased calcium deposition compared to bare scaffolds [62]. In vivo testing showed greater bone formation. Gao et al. used thermal inkjet printing to study osteogenesis of printed BMSCs in polyethylene glycol demethacrylate, bioactive glass, and hydroxyapatite (HA) [36]. The HA group showed the highest cell viability and compressive modulus at 21 days as well as the most collagen production and highest alkaline phosphatase activity. Patel et al. also suggested that the addition of HA enhances BMSC differentiation by promoting endogenous osteogenic signals [63]. In vivo study of 3D-printed polylactic acid/HA scaffolds seeded with BMSCs showed they have good osteogenic capability with no difference in inflammation (Fig. 3B) [64].

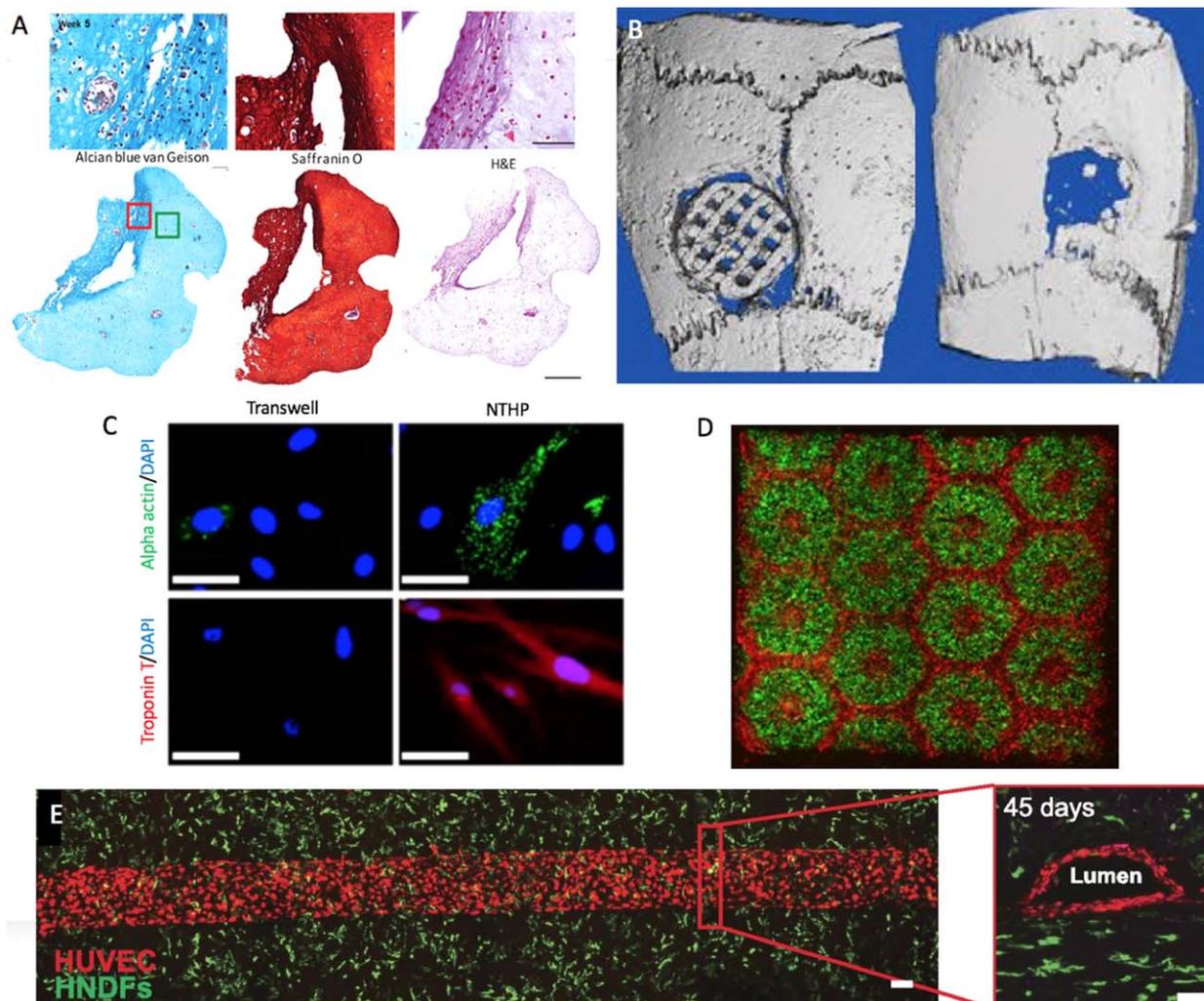


Figure 3. Bioprinted tissues. **(A):** 3D-bioprinted chondrocyte-derived iPSCs at week 5 of differentiation, sections stained for GAGs, Safranin O for cartilage (with nuclear counterstain), and H&E for extracellular matrix (with nuclear counterstain) (the scale bar represents 100 μm or 500 μm) (Reproduced with permission from [58]). **(B):** Micro-CT images of poly(lactic acid/hydroxyapatite) scaffold (left) versus bone defect without scaffold (right) after 4 weeks in vivo. (Adapted and reproduced with permission from [58]). **(C):** Immunocytochemistry of MSCs for cardiac proteins in the transwell versus nanothin and highly porous membrane methods (Adapted and reproduced with permission from [59]). **(D):** 3D bioprinting of hydrogel based hepatic construct. Images ($\times 5$) showing patterns of fluorescently labeled hiPSC-HPCs (green) in 5% (wt/vol) GelMA and supporting cells (red) in 2.5% (wt/vol) GelMA with 1% GMHA on day 0 (Scale bars, 500 μm ; Reproduced with permission from [60]). **(E):** Top-down (left) and cross-sectional (right) confocal microscopy images of bioprinted vascular networks supporting human neonatal dermal fibroblast-laden (green) matrix and HUVEC (red) lined channels obtained after 45 days of perfusion culture. (Scale bar: 100 μm ; Reproduced with permission from [61]). Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; H&E, hematoxylin and eosin; HNDFS, human neonatal dermal fibroblasts; HUVECs, human umbilical vein endothelial cells; NTHP, nanothin and highly porous.

Cardiac Muscle

Coronary artery disease is a leading cause of morbidity and mortality in the U.S. [65]. Bioprinted cardiac patches can be used to help heal damaged myocardium after a heart attack. Human cardiac derived cardiomyocyte progenitor cells (hCPCs) were printed with alginate and had 92% viability after 1 day and 89% after 7 days [35]. They also retained their commitment to a cardiac lineage with enhanced gene expression of early transcription factors, suggesting they could be used in cardiac tissue engineering. Gaebel fabricated a cardiac patch using laser-induced-forward-transfer (LIFT) consisting of polyester urethane urea with human umbilical vein endothelial cells (HUVECs) and MSCs [66]. The patches were transferred into infarcted rat hearts and increased vessel formation as well as functional improvement was seen in

the LIFT group. Another group used dECM-based bioink with hCPCs or turbinate MSCs to fabricate cardiac patches [67]. Patterned patches (containing both cell types) reduced cardiac hypertrophy and fibrosis, increased migration to the infarcted area, and improved neo-muscle and capillary function in vivo. Nanothin cell sheets can also be printed with various cells in different layers. Human BMSCs and rat cardiomyocytes were cocultured on a printed cellulose acetate membrane and remained highly viable and transferable suggesting that this platform would be effective for therapeutic use (Fig. 3C) [59].

Neural Tissue

Peripheral nerve injuries are common after trauma with 1.4 million injuries occurring per year in the U.S. [68]. The gold standard

for a nerve conduit is use of an autograft; however, this has limitations including donor site morbidity. Due to this, a number of approaches have been studied including bioprinting. Owens et al. used multicellular cylindrical units composed of BMSCs and Schwann cells to fabricate fully cellular bioprinted nerve grafts [69]. They demonstrated the recovery of both motor and sensory function with long-term functionality at 40 weeks in a rat sciatic nerve injury model. Other groups have worked on creating functional neural tissues. Gu et al. used a novel alginate, carboxymethyl-chitosan, and agarose bioink with neural stem cells and showed the differentiated neurons form synaptic contacts, establish networks, are spontaneously active, and show increased calcium response to bicuculline [28]. Another group printed neural stem cells in thermoresponsive biodegradable polyurethane and studied the constructs in a zebrafish traumatic brain injury model [70]. Function was improved after implantation of one form of the constructs, suggesting this bioink may offer new possibilities in neural tissue engineering.

Liver

Many patients with end-stage-liver-disease die waiting for an organ because donors are scarce, leading to alternative strategies for liver replacement. One group bioprinted iPSCs and differentiated them into hepatocytes showing that stem cells can maintain their pluripotency after bioprinting [71]. Another group printed ADSCs and were able to successfully convert them into a hepatogenic lineage with expression of liver genes [31]. A new cross-linking system was used in their study, which improved gene expression profiles. Ma et al. successfully printed a hexagonal structure of liver cells and supporting cells (Fig. 3D) [60]. Other groups have developed liver models with organized hepatic structures which may be used for drug testing [72]. These organs-on-a-chip will likely be the first successes in organ bioprinting [73].

Vasculature

Vasculature network formation is a major limiting factor in the creation of scaled-up tissues and organs. Constructs larger than 200–400 μm require vasculature, as this is the maximum diffusion distance [74]. All three printing modalities have difficulty creating complex hollow structures. One approach to create vasculature is to use a sacrificial material. Initially agarose was used and it was removed with a vacuum, but this compromised channel structures [75]. Therefore, other groups have used Pluronic, which is a solution below 4°C. Kolesky et al. printed Pluronic channels and endothelialized them with HUVECs [39]. They showed that the bioprinted vasculature remains stable during long-term perfusion (45 days) [61]. On the other hand, direct bioprinting can be used. Dolati et al. used a coaxial nozzle to print scaffold free perfusable vascular conduits which were mechanically strong and cell viability was high [76]. Since capillaries are too small to be printed, angiogenesis is relied on to create fine interconnections between bioprinted microvascular channels (100 μm ; Fig. 3E) [77].

TRANSPLANTATION OF BIOPRINTED TISSUES

Challenges and Requirements

Surgical challenges similar to allogenic organ transplants including cellular ischemia, vascular anastomoses, and size match will persist. However, ischemia time should be limited since the organ can be perfused in laboratory settings. Any construct greater than 100–200 μm requires vascularization [73]. Fabrication of an organ

with vascularity is a major area of research currently. Additionally, size match can be planned pre-operatively with imaging such as CT scans and CAD. The implanted tissue or organ must consist of biocompatible materials that integrate with native cells, allow for vascular ingrowth, and avoid immune response. Many ethical dilemmas and regulatory issues will likely develop as this technology progresses [78].

Immune Acceptance

Patients who receive allogenic organ transplants are subjected to lifelong immunosuppression to prevent immune rejection. These drugs have many associated side effects including increased infection and cancer risk. Their levels must be monitored regularly and sometimes they do not prevent rejection, subjecting patients to prolonged steroid courses and hospitalizations. Steroids have additional side effects of hyperglycemia, weight gain, osteoporosis, and decreased muscle function [79]. Adult stem cells offer the ability for autologous tissue production which may avoid the need for immunosuppression. In addition, supporting materials and bioink components will need to be biocompatible with low inflammatory response to prevent tissue inflammation and macrophage recruitment.

Monitoring

Traditional organ transplant and free flap patients are initially placed in intensive care units for close monitoring postoperatively. This will likely remain a necessity as the surgeries will be similar in technique and duration, therefore leading to similar physiology and patient monitoring requirements. Even though immunosuppression will likely not be needed, the grafts will still be monitored for rejection. Overall organ function can be monitored with laboratory testing or imaging. For example, blood urea nitrogen, creatinine, and electrolytes can be used to monitor kidney function or cardiac enzymes (creatinine kinase and troponin) and echocardiogram to monitor heart function. Biomedical devices or implants also have an increased infection risk with increased morbidity and mortality [80]. Any sign of infection would require prompt initiation of antimicrobial therapy.

FUTURE PERSPECTIVES

One of the major hurdles to fabrication of large scale organs for transplantation is the incorporation of vasculature at multiple scales [81]. Macroscale vasculature will be needed for surgical anastomosis and creation of this will likely be accomplished by advances in bioprinting techniques. These larger vessels will then need to be integrated with microscale capillaries. Since direct bioprinting of capillaries is not feasible due to current resolution of bioprinting techniques, microvascular channels can be bioprinted with adjacent endothelial cells to allow angiogenesis to create capillary networks. In addition, growth factors can be incorporated in the media to enhance angiogenesis.

Before clinical application, stem cell processing must be standardized as well as the quality of stem cells used. There are currently several different processes, which are still evolving. As native tissues and organs are made of multiple cell types, robust and efficient stem cell differentiation protocols are required. The lack of standardized differentiation protocols leads to varying results between groups. Additionally, differentiation requires prolonged culture periods leading to risk of contamination and use of antimicrobials and antimycotics. Human forms of all serum

reagents used in cell culture must be developed to prevent transfer of zoonoses. Core manufacturing facilities for stem cell processing and tissue biofabrication must be developed and isolation facilities will need to be created in clinics in order to facilitate transition into clinical use. Bioprinted tissues will face many regulatory hurdles as they will likely be monitored as a device, biologic, and/or drug by the Food and Drug Administration.

CONCLUSION

There have been many advances in bioprinting technologies over the past several decades, including scaled-up tissues and integration of vascularization. Multiple tissue types have been fabricated from both primary and stem cells including cartilage, bone, cardiac muscle, neural tissue, liver, and vasculature. However, many hurdles must still be overcome before wide-scale clinical applicability is achieved. Technical challenges of creating human-scale tissues with physiologically-relevant vasculature and cell distributions in addition to economic and ethical obstacles currently limit clinical implementation. Nonetheless, these novel technologies have great potential to fabricate replacement tissues and organs in the future. Organ-on-a-chip models will likely serve as an intermediate step toward the creation of large scale vascularized organs.

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AUTHOR CONTRIBUTIONS

A.N.L.: literature review and analysis, manuscript writing; D.J.R.: manuscript writing, financial support; A.D.: manuscript writing; I.T.O.: conception and design, final approval of manuscript, financial support.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

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