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Additive Manufacturing of Biomaterials, Tissues, and Organs

State-of-the-Art Review of 3D Bioprinting for Cardiovascular Tissue Engineering

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Abstract—3D bioprinting is a group of rapidly growing techniques that allows building engineered tissue constructs with complex and hierarchical structures, mechanical and biological heterogeneity. It enables implementation of various bioinks through different printing mechanisms and precise deposition of cell and/or biomolecule laden biomaterials in predefined locations. This review briefly summarizes applicable bioink materials and various bioprinting techniques, and presents the recent advances in bioprinting of cardiovascular tissues, with focusing on vascularized constructs, myocardium and heart valve conduits. Current challenges and further perspectives are also discussed to help guide the bioink and bioprinter development, improve bioprinting strategies and direct future organ bioprinting and translational applications.

Keywords—Bioink, Hydrogel, Vascularization, Heart valve, Organ Bioprinting.

INTRODUCTION

Cardiovascular disease (CVD) represents the leading causes of worldwide morbidity and mortality, approaching 20 million deaths annually.[28] As fully differentiated and load bearing tissue, cardiovascular tissues, including heart valves, arteries and myocardium, usually end up with replacement at the end of disease stage. Annually, more than 80,000 heart valve replacements in the United States alone[56] and over 600,000 vascular implantations are performed,[85] resulting in approximate expenditure of US$200 billion.[28,95] Current treatment strategies include autografts (e.g., coronary artery bypass graft with autologous vein, Ross procedure), allografts (donor valve or heart transplantations), xenografts (bovine or porcine heart valves, arteries *et al.*) and artificial prostheses (biopolymer vascular grafts, mechanical valves, cardiac assist devices). However, each of these approaches has its own disadvantages, which include, but not restricted to, donor tissue shortage, immune rejection, anticoagulation therapy, and limited durability.[7] The emerging field of tissue engineering and regenerative medicine holds great promises as an approach for creating engineered tissues to repair congenital defects (like aortic valve stenosis and coarctation of the aorta) and/or diseased cardiovascular tissues.[32,88]

As one of advanced fabrication techniques, 3D printing, also referred as additive manufacturing (AM) or solid free form fabrication, employs automated processes and standardized materials as building blocks and enables creation of 3D objects from personalized specific computer-aided designs.[20,59] 3D printing has already been used to generate individualized models for cardiovascular surgeons to visualize anatomical structures.[27,87] Different from traditional artificial heart models and cadaveric models, the printed models can facilitate better understanding of structural abnormalities and choosing better surgical approaches. NIH also launches 3D printing exchange, which is a public website that enables users to share, download and edit 3D print files related to health and science. Mostly, these surgical models are made of solid plastic and are not directly applicable for tissue engineering purposes.

The 3D printing approach has been introduced into tissue engineering field by using biodegradable biopolymers for building complex and composite

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scaffolds and tissues constructs.[77] Many pioneering works have been widely reported on using printable biomaterials and 3D printing approaches for scaffold fabrication with post cell seeding, conditioning and even *in vivo* implantation.[19,93,99,103] Several comprehensive reviews have also been published summarizing working mechanism, biomaterial choices and tissue engineering applications.[49,60,101] For these printing approaches, surface cell seeding is required and the application is sometimes limited due to the difficulties in incorporation of multiple cell types and bioactive molecules within the constructs due to high temperature and laser energy.[21,96] Recently, by combining AM and living cells/biological factors, bioprinting has been gained more and more attention.[14,71] This technique can be defined as the use of computer-aided layer-by-layer deposition approach for patterning and assembling living cells and biologics within a prescribed 2D or 3D constructs.[30] Comparing to other biofabrication techniques, bioprinting allows the production of 3D constructs with precisely controlled architecture, multiple cell types and more physiologically relevant microenvironments.[65]

This review aims to provide a state-of-the-art overview of implementation of 3D bioprinting techniques for cardiovascular tissue engineering. First, it briefly presents some related backgrounds about bioprinting working principles, applicable bioink materials and process configurations. Then I focus on recent advances in bioprinting of vascularized constructs, blood vessels, and myocardium and heart valves. Finally, current major challenges and technological hurdles are discussed and potential solutions and future directions are provided.

BACKGROUND

Similar to normal AM (3D printing) techniques, 3D bioprinting produces complex objects from a 3D design file by decomposing the shape into series of 2D layers. The 3D bioprinter deposits bioinks (building blocks containing cells/biomaterials mixture or spheroids) in a layer-by-layer manner based on the design. Each layer is then bonded to the previous layer to fabricate the 3D constructs.[14,62] Bioprinting technology also allows the fabrication of biomimetic and even anatomical shaped 3D structures by using patients' images obtained from medical imaging technologies (e.g., computer tomography-CT and magnetic resonance imaging-MRI).[65,69]

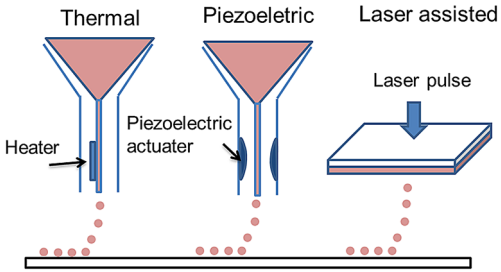
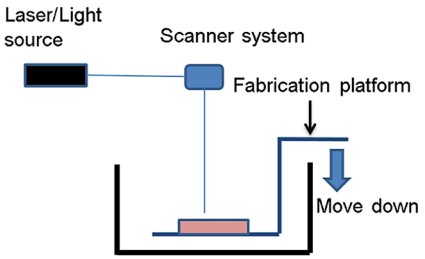
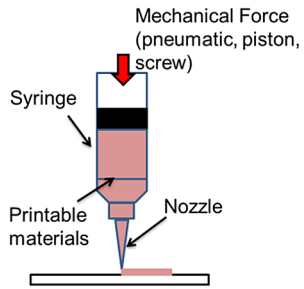
Comparison of Different Bioprinting Techniques

Currently, several types of bioprinting techniques, including inkjet, laser/light, and extrusion based bioprinting, have been used. Inkjet based bioprinting implements different mechanisms (thermal,[13] piezoelectric actuator,[83] laser-induced forward transfer,[31] and pneumatic pressure[10]) to generate small bio-ink droplets onto a substrate. This technique produces relatively high resolution patterns and is more suitable to generate thin layers[9,39] or patterned structures[1] for soft tissue regeneration or for single/multiple cell manipulation. Laser/light based bioprinting (stereolithography/projection bioprinting) implements laser or other light sources (like UV or near-infrared light) to scan over the surface of photocurable polymer solution.[40] Then the stage lowers incrementally, allowing layers to be polymerized on top of each other, thus creating 3D structures in a bottom-up manner.[104] Extrusion based bioprinting (EBB) utilizes mechanical force driven by air pressure and motor to extrude biomaterials (normally hydrogels), cell aggregates or microcarriers through a nozzle in a controlled manner to construct a 3D structure. The typical diameter of the nozzle is about 150–300 μm to minimum the cell damage.[45] Table 1 summarizes and compares the advantages and disadvantages of different bioprinting techniques.

Bioinks

Various bioinks have been used in bioprinting, including cell suspensions (for inkjet based), cell-laden hydrogels, microcarriers, cell/tissue spheroids and decellularized matrix components.[6,52,75] For inkjet and laser based bioprinting, the choices of bioinks are limited, as previously mentioned, due to required printing process. Generally, the inkjet and laser based bioprinting require liquid-like bioinks, while EBB requires bioinks with certain viscosity. Hydrogels are the most widely used bioink for EBB and the choice of hydrogels should be matched with the type of regenerated tissue. For example, biostable hydrogels with limited or slow biodegradability and higher mechanical properties like poly(ethylene glycol) (PEG) based hydrogels, alginate, agrose and methylcellulose are more often used for bioprinting of cartilage.[26,44,57] Bioactive hydrogels such as gelatin, collagen, fibrin and peptide with capacity to support cell adhesion are usually implemented for cardiovascular bioprinting.[3,5,55,70] Microcarriers offer a high specific surface area and bioactive environment for quick cell attachment and proliferation.[52] Cells can be encapsulated within microcarriers and further incorporated within bioinks for bioprinting. Scaffold-free cell spheroids generated by biofabrication approaches like hanging drop, micro-molded, microfluidics, and spinner flasks are also used in bioprinting. The deposited spheroids can fuse together and quickly generate into more ma-

TABLE 1. Comparison of different bioprinting techniques.

Bioprinting Category		Pros	Cons	Ref
Inkjet based		Produce relatively high resolution patterns (picolitre to microliter in volume, up to several micrometer spatial resolution) More suitable to generate thin layers or patterned structures Support printing with multiple cell types	Prolonged printing time due to small volume of printed droplets Restricted to low viscous biomaterials solutions Weak mechanical properties	1,10,12,83
Laser/light based		Generate 3D constructs with relatively high resolution (depending on laser and light sources, up to several micrometers) Capable of incorporating living cells by carefully choosing light source and optimizing printing conditions to minimize potential cell damage	Normally have small size and are not clinically applicable Implementation of laser/light significantly increases the complexity of the bioprinting system	29,84
Extrusion based		Faster and more controllable deposition and printing speed Generate 3D constructs with clinical relevant size and anatomical shape in a relatively short period of time Supports wider range of bioinks and enables higher cell density and lower cell damage compared to laser based bioprinting	Lower resolution (usually over 100 μm Not suitable for precise cell patterning or organization Require viscous bioinks and limit the usage of hydrogel types Affect cell viability due to gelation/solidification process and shear thinning	6,46,70

ture constructs with heterogeneous cell population and better biomimicry. Therefore, this enables co-culture of endothelial cells, smooth muscle cells, fibroblasts, cardiomyoctyes and/or other related cardiovascular cells types. However, the whole bioprinting process, including generation of a huge number of spheroids, spheroid loading, deposition and construct handling, is labour-intensive and time consuming process, which limits its application.[94] In addition, the scaffold-free cell spheroid based constructs are mechanically weak and require long time to get remodelled and fully mature. Apart from cell/tissue spheroid, extracellular matrix (ECM) from various native tissues is also considered as a new bioink source. In general, ECM is first decellularized and then dissolved/concentrated into pasty-like bioinks after chopping and smashing.[74,75] This approach provides almost unlimited bioink sources and more native-tissue like microenvironments. However, the decellularization process should be standardized based on tissue sources to make consistent and component controllable bioinks. Sometime, hydrogel based bioinks are combined with decellularized ECM bioink or a supportive frame is printed first to improve the mechanical properties and bioprintability. However, the hybrid printing strategy increases the complexity and requires better software and hardware control.

3D BIOPRINTING OF MICROVASCULATURES AND VASCULARIZED CONSTRUCTS

Functional vascular network is essential to facilitate oxygen transfer, deliver nutrients, remove metabolic waste and promote the circulation of immune cells.[90] It plays a crucial role in regeneration of cardiovascular tissues and other tissues, like bone, liver and pancreas, which are highly vascularized.[38,80] Although tissue engineering aims to create functional tissues and even organs for decades, current strategies still cannot generate fully vascularized tissue constructs, which limit many tissue engineering applications. 3D bioprinting adopts vascularization strategies from general tissue engineering approaches and combines with its own advantages to create *in vitro* vasculature and vascularized constructs. These approaches include (a) generation of vascular constructs by self-assembly of cells; (b) generation of microvasculatures by inkjet based bioprinting; (c) generation of bioprinted constructs with growth factor delivery; (d) coaxial nozzle-assisted 3D bioprinting of vasculature and (e) generation of channel based vascularized constructs.

Cell self-assembly is the autonomous organization of cells with similar adhesive properties into a stable pattern or structure without external intervention.[41] Cell spheroids prepared from cell suspensions were implemented as building blocks and the deposited spheroids fused into vascular like constructs (Fig. 1A).[63] With the application of agarose rods as templates, tubular structures with controllable tube diameter, wall thickness and even branching pattern can be achieved by deposition and fusion of multicellular spheroids (Figs. 1B and 1C).[67] In addition, double-layered vascular wall and specific pattern can be achieved by alternatively depositing multicellular cylinders composed of human smooth muscle cells (HSMC) and human skin fibroblasts (HSF) (Fig. 1D).[67] The formation of large amounts of these building blocks is usually time consuming and requires a lot of manual work.[58] By using optimized computer aided algorithms and support hydrogels, Kucukgul *et al*. 3D bioprinted mouse embryonic fibroblast (MEF) cell aggregates, from cell suspension rather than cell spheroids, to form an aortic tissue construct.[48] This approach is relatively more efficient, but the overall resolution and controllability need to be further improved.

For inkjet based bioprinting, commercial inkjet printer has been modified by Cui *et al*. to simultaneously deposit human microvascular endothelial cells (HMEC) and fibrin to form the microvasculature (Fig. 1E).[12] Printed fibrin scaffold retained proper shape after printing (Fig. 1F) and endothelial cells proliferate to form a tubular structure. The printed ring shaped microvascularture had much better integrity after 21-days culture, excluding Texas Red conjugated dextran from the printed structure (Fig. 1G). Again, the inkjet based bioprinting enables multiple cell types and the use of mesenchymal stem cells or smooth muscle cells can support the formation and maturation of microvasculatures. However, all the bioprinting and cell deposition are normally performed on a substrate (bio-paper) to support the weak cell layer(s).

Angiogenic growth factors also play an important role in neovascularization. They can activate the endothelial (progenitor) cells, regulate their migration and promote cell assembly, vessel formation and maturation.[68] In order to achieve effective delivery, temporal and special delivery strategies, like nanoparticles and microspheres, are used instead of direct addition *in vitro* and injection *in vivo*. Poldervaart *et al*. investigated the controlled release of vascular endothelial growth factor (VEGF) from gelatin microparticles (GMP) within 3Dbioprinted scaffolds, and the effects on subsequent vascularization.[78] VEGF was first incorporated into the Matrigel (fast release) or into the GMP which were then dispersed in Matrigel plugs (slow release). Combined application of GMP embedded in Matrigel plugs showed significant pro-

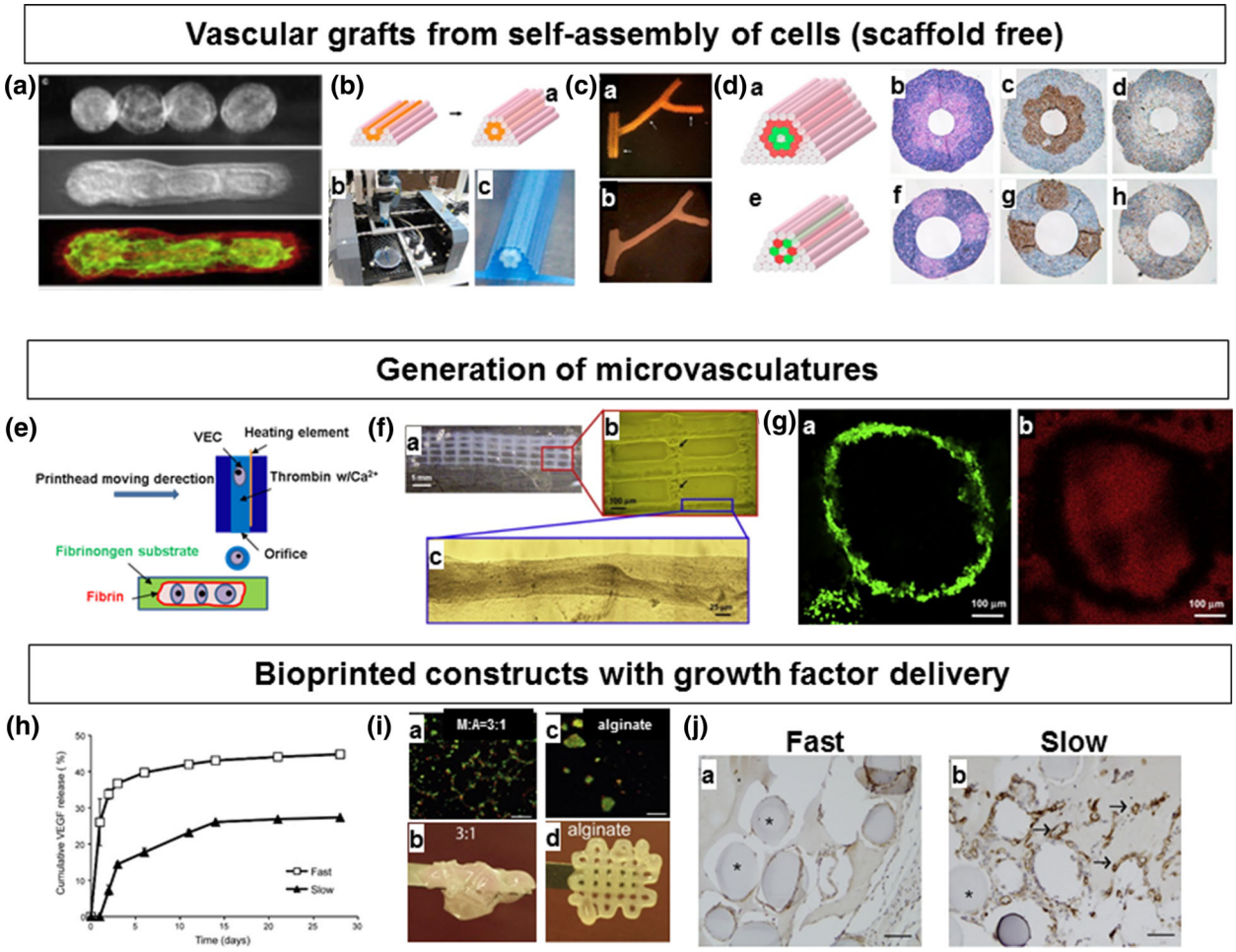


FIGURE 1. Strategies to generate microvasculatures and vascularized constructs. Generation of vascular constructs by self-assembly of cells. (A) Sequential steps of cell fusion of vascular tissue spheroids in collagen I hydrogel[63]; (B) bioprinted tubular structures with cellular cylinders[67]: (a) design template, (b) bioprinter with two vertically moving print heads, (c) printed construct; (C) Fusion pattern of multicellular spheroids assembled into branched structure[67]: (a) built of 300 μm spheroids with branches of 1.2 mm (solid arrow) and 0.9 mm (broken arrows), (b) fused branched construct after 6 days of deposition; (D) built of a double-layered vascular wall[67]: (a, e) multicellular cylinders assembled by SMC (green) and fibroblasts (red), (b, f) H&E staining, (c, g) α smooth muscle actin (brown), (d, h) Caspase-3 (brown). Generation of microvasculatures by inkjet based bioprinting.[12] (E) Schematic drawing of simultaneous deposition of HMEC and fibrin channel scaffold using modified thermal inkjet printer; (F) printed fibrin scaffold; (G) printed ring shaped microvasculature, (a) cultured for 21 days (calcein AM, green), (b) improved integrity after 21-day culture with all dextran molecules (red) excluded from the printed structure. Generation of bioprinted constructs with growth factor delivery.[78] (H) Cumulative release of VEGF. Fast (directly incorporated in Matrigel) and slow release (application of gelatin microspheres) of VEGF; (I) bioprinted hydrogel mixture and tubulogenesis assay; (J) vessel formation in EPC seeded scaffolds after one week subcutaneous implantation in mice: (a) fast release showed less CD31 (brown) than (b) slow release of VEGF in the bioprinted hydrogels (Matrigel:alginate = 3:1). Blood vessels are indicated with arrows. Coaxial nozzle-assisted 3D bioprinting of vasculature. (K) Schematic of fabrication of a 3D alginate structure with built-in microchannels[25]; (L) 3D construct fabricated based on hollow alginate filaments[25]: (a) printed construct, (b) longitudinal section, (c) SEM image of the cross section; (M) printed alginate based vasculature[102]: (a) in the perfusion chamber under pulsatile flow, (b) with zigzag shape, (c) H&E staining showed collagen and smooth muscle deposition after 6-week culture. Generation of channel based vascularized constructs. (N) Formation of printed carbohydrate-glass filament-architecture and vascular lumen with endothelial monolayer after removing sacrificial filament and perfusion[61]; (O) (a) gelatin based constructs with branched bioprinted agarose templates, (b) the microchannel promoted cell viability around it (calcein AM, green; ethidium homodimer-1, red)[4]; (P) (a, b) schematic views of heterogeneous engineered tissue construct, (c) printed channel, (d) evacuation of sacrificial Pluronic F127, (e) fluorescent image of three cell types within 3D printed tissue construct[47]; (Q) (a) formation of dual channel by bioprinting sacrificial gelatin within fibrin, (b) GFP-HUVEC (green) within fibrin showed tube structure and capillary network after 12-day culture and RFP-HUVEC (red) in the channel developed lumen structure.[51] Reproduced with permission from Refs. 4,12,25,47,61,63,67,78,102.

longed VEGF release (Fig. 1H). The addition of alginate into Matrigel enhanced the mechanical properties and 3D bioprintability, but induced large cell aggregates and reduced tubulogenesis of endothelial progenitor cells (EPC) (Fig. 1I). Heterogeneous 3D bioprinted scaffolds consisted of a mixture of Matrigel/alginate = 3/1 with VEGF were then implanted subcutaneously in mice. It was found that slowly released

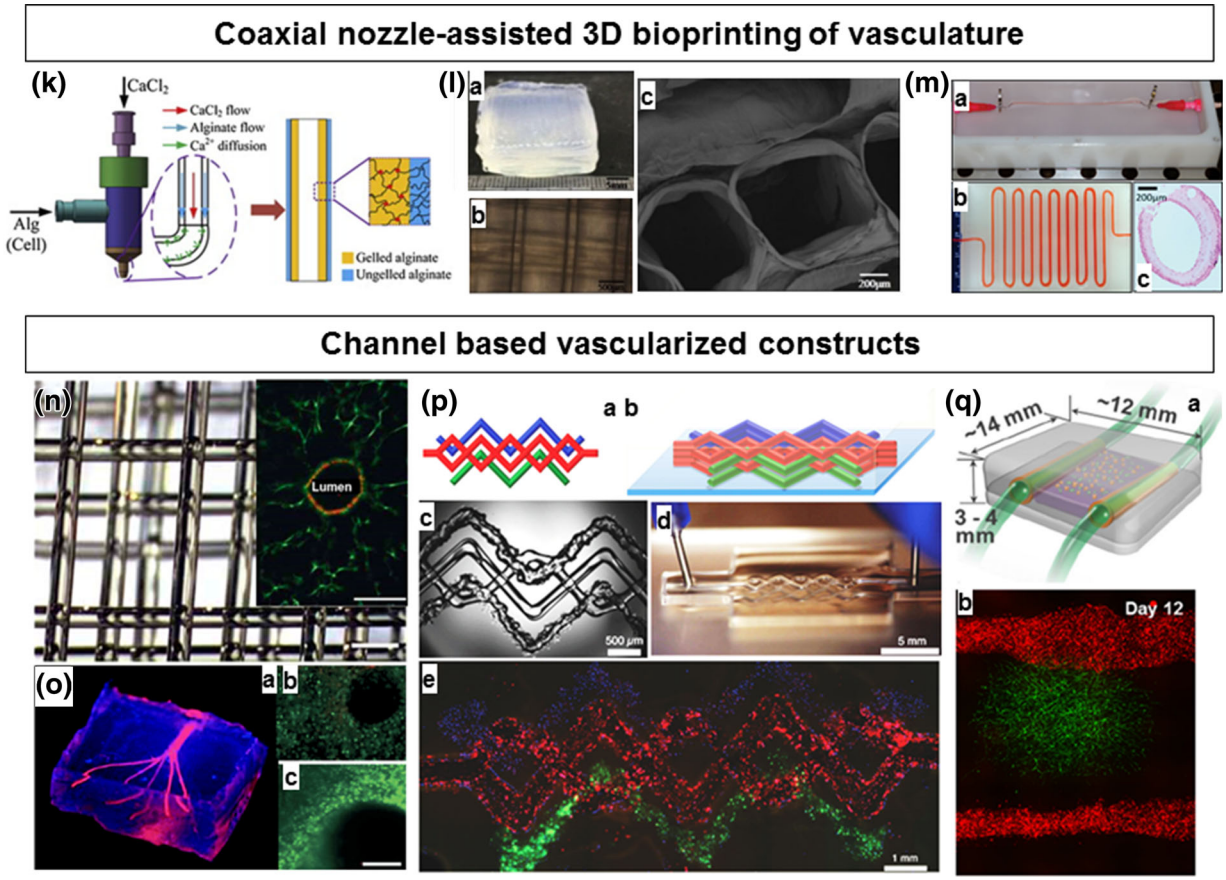


FIGURE 1. continued

VEGF promoted more vessel formation, with more CD31 expression, comparing to fast release counterparts (Fig. 1J). The nano-/microparticle delivery strategies enable dual or even multiple release of therapeutic reagents and angiogenic factors in a synergetic way within bioprinted constructs.[72]

Both micro- and macro-engineered cardiovascular tissues require vascular network to maintain cell viability and meet oxygen and nutrient demands. Large clinical relevant cardiovascular constructs also need flow throughout the entire construct. Unfortunately, angiogenic factors and endothelial cells cannot provide immediately flow and generate perfusable constructs within short time.[76] 3D bioprinting provides a great opportunity to produce controlled vascular networks with clinical relevant size, perfusable channels, and multiple cell types. Gao *et al*. implemented a coaxial nozzle-assisted 3D bioprinting system to fabricate hollow calcium alginate filaments.[25] The sodium alginate solution (with or without cells) dispensed through the outer tube of the coaxial nozzle get crosslinked when contact calcium chloride solution in the inner of the coaxial nozzle and form the filament with a hollow channel (Fig. 1K). The hollow alginate filaments were then used as the building blocks for further printing (Fig. 1L). Scanning electron microscopy image (SEM) confirmed the formation of hollow structure and the uniform fusion section between adjacent hollow filaments (Fig. 1L). Similarly, Yu *et al*. generated alginate based vasculatures using similar bioprinting setup.[102] The vasculatures can be printed at defined geometry, length, and orientation (Fig. 1M). With encapsulation of human umbilical vein smooth muscle and pulsatile flow, deposition of collagen was observed after 6-week culture (Fig. 1M). The major disadvantage of coaxial nozzle-assisted 3D bioprinting is the limited availability of bioink. Currently, only alginate based bioink is used due to its fast ionic crosslinking capacity and other bioinks are hardly used in this setup.

In addition to print the channel directly, many research groups are printing sacrificial materials to generate channel networks within engineered tissue constructs. This process involves 3D printing of water soluble material based networks into bulk materials (either bioprinted or casted, typically cell-laden hydrogels). Then the soluble channel network is dissolved, commonly by solution or changing temperature. Miller *et al*. printed rigid 3D filament networks of

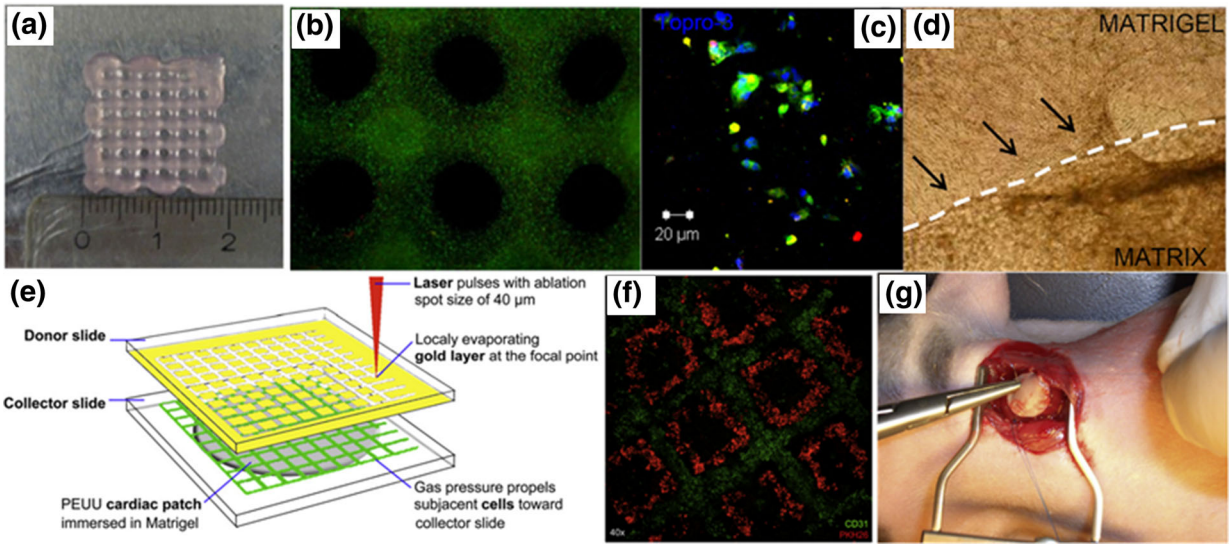


FIGURE 2. 3D bioprinting of myocardium. Extrusion based bioprinting of scaffolds with hCMPC.[23] (a) Bioprinted scaffolds, (b) high cell viability of hCMPC, (c) expression of human β-integrin and Ki-67, (d) migration of hCMPC after 3-week culture. Inkjet based bioprinting of cardiac patch.[22] (e) Schematic bioprinting setup, (f) patterned cells, (g) patch implantation *in vivo* in a rat model. Reproduced with permission from Refs. 22,23.

carbohydrate glass, and used them as a cytocompatible sacrificial template to generate cylindrical networks (Fig. 1N).[61] The sugar-glass networks are compatible with many types of cell-laden matrices and the formed channel networks after removing sugar-glass can support endothelial cells and pulsatile flow of human blood with intervessel junctions supporting branched fluid flow (Fig. 1N). Bertassoni *et al.* in Khademhosseini lab reported utilizing bioprinted agarose template fibers to fabricate perfusable microchannel networks within gelatin based hydrogel constructs.[4] The fabricated vascular networks improved mass transport, cellular viability and differentiation within the cell laden tissue constructs (Fig. 1O). Kolesky *et al.* bioprinted both sacrificial materials (Pluronic F127) and multiple cell-laden hydrogels (methacrylated gelatin) to form perfusable networks.[47] To demonstrate perfusable channels printing and patterning of multiple cell types, they used four print heads to first print a PDMS border and then print sacrificial Pluronic F127 with two different Gel-MA inks containing fluorescent labelled fibroblasts (Fig. 1P). After removal of Pluronic, microchannels were endothelialized with RFP human umbilical vein endothelial cells (HUVEC) (Fig. 1P). Lee *et al.* generated constructs with fluidic vascular channels (lumen size of ~1 mm) by sandwiching printed sacrificial gelatin into printed collagen matrix.[50] The fluidic vascular channel can support the viability of tissue up to 5 mm in distance at 5 million cells/mL density under the physiological flow condition. The same group also created two fluidic vascular channels and deposited fibrin-cell mixture in the middle (Fig. 1Q).[51] HUVEC transfected with green fluorescent protein (GFP, green) and mCherry (red) were separately cultured and used for fibrin gel and fluidic channels, respectively (Fig. 1Q). The HUVECs began to form tube structure after 1-week culture, and the capillary network became denser, created more branches with lumen.

3D BIOPRINTING OF MYOCARDIUM

The most causes of death within CVD is ischemic disease (e.g., myocardial infarction-MI also known as heart attack), which represents 42% (7.3 million) of all CVD deaths.[28,100] Acute MI is normally caused by the block of one of the coronary arteries and the lack of blood flow consequently results in ischemia (lack of oxygen). If the blood flow is not recovered quickly, cardiomyocytes (CM) die within the blood-deprived myocardium. Fibroblasts/myofibroblasts and endothelial cells migrate and gradually form noncontracting fibrotic scars after a vigorous inflammatory response is provoked. Scar formation with little myocardial tissue reduces contractile function of the heart ultimately leading to heart failure. A great number of cells is lost upon injury and CM rarely divide.[66] Heart transplantation is the last option for severe heart failure, but this strategy is restricted due to donor organ shortage and rejection. Cellular cardiomyoplasty, or cell-based cardiac repair, has been made remarkable progress in myocardial tissue regeneration.[79] This approach involves injecting cells

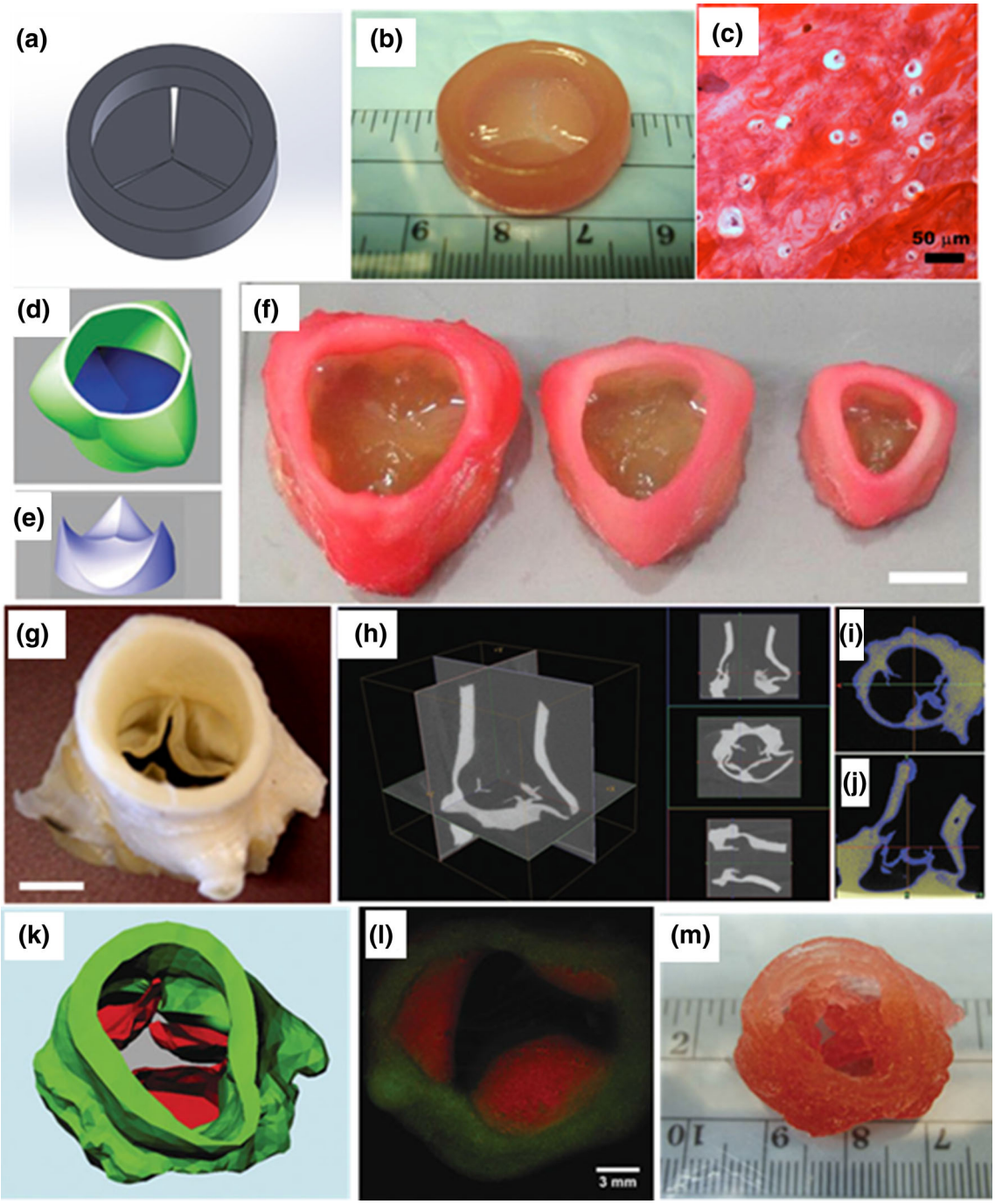


FIGURE 3. 3D bioprinting of heart valve. (a–c) Flat valve[18]; (d–f) axisymmetric valve[37]; (g–m) anatomical valve.[16,36] (a, d, e, k) Valve model; (b, f, m) bioprinted valve; (c) Safranin-O staining showed GAG deposition; (h) μCT scan slices and their reconstruction; (i, j) the valve scans were viewed, thresholded, and segmented into separate STLs for the leaflet and the root; (l) fluorescent image of first printed two layers of aortic valve conduit. Reproduced with permission from Refs. 16,18,36,37.

into myocardium, which is surgically less invasive, but the injected cells have low viability and hardly integrate with host cells.

Myocardial tissue engineering (MTE) requires high density of CM and various supporting cells, vascularization and efficient oxygen exchange to generate synchronous contractions.[35] 3D bioprinting can pattern and assemble cells with high density, defined organization and spatial distribution. It also enables the generation of multiple layered constructs with multiple cell types. Gaetani *et al.* implemented EBB and bioprinted alginate and RGD-modified alginate scaffolds

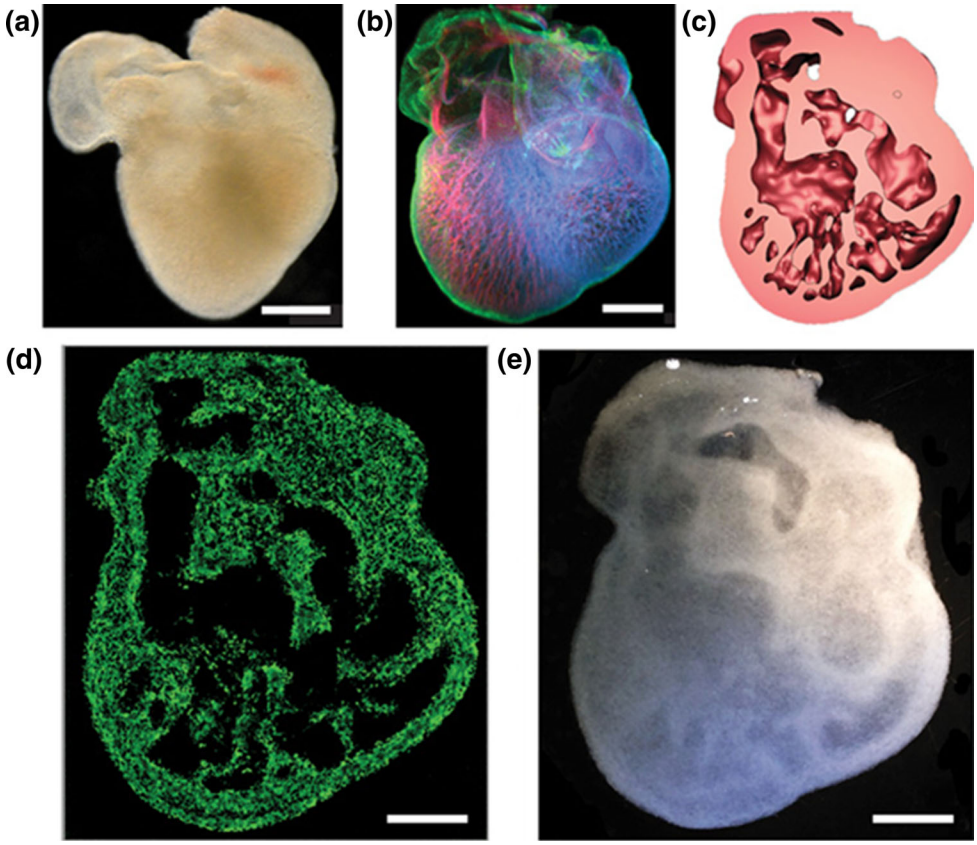


FIGURE 4. 3D bioprinted scaffolds based on 3D imaging data from whole heart.[34] (a) Image of explanted embryonic chick heart; (b) 3D image of the embryonic chick heart stained for fibronectin (green), nuclei (blue), and F-actin (red); (c) cross section of the 3D CAD model of the embryonic heart; (d) cross section of the 3D printed heart (fluorescent alginate-green); (e) 3D printed heart with internal structure visible through the translucent heart wall.

with human fetal CM progenitor cells (hCMPC) (Fig. 2a).[23] They demonstrated that printed hCMPCs had high cell viability, retained their commitment for the cardiac lineage and expressed enhanced gene expression of the early cardiac transcription factors and the sarcomeric protein TroponinT within 3D culture (Figs. 2b and 2c). The bioprinted cells were also able to migrate from the constructs and formed tube-like structure on the Matrigel layer (Fig. 2d). Gaebel *et al.* applied the laser induced inkjet bioprinting technique and patterned HUVEC and human mesenchymal stem cell (hMSC) on a polyester urethane urea cardiac patch (Fig. 2e).[22] Specific vascular patterns were successfully generated and HUVEC (green) and hMSC (red) arranged in a capillary like pattern (Fig. 2f). Patches with patterned and randomly seeded cells were cultivated further transplanted *in vivo* to the infarcted zone of rat hearts after left anterior descending (LAD)-ligation (Fig. 2g). The same group further bioprinted patch composed of hCMPCs laden HA/Gel matrix.[24] Similarly, hCMPCs retained their cardiogenic phenotype in the bioprinted constructs up to 1 month and the patch preserved heart function by reducing LV remodelling and improving myocardial viability.

Similar to standard MTE approaches, it is essential for 3D bioprinting of thick muscle-like tissues to generate synergistic contractile force and to adequately repair or replace the damaged heart tissue. We are facing difficulties to functionally integrate the graft and the host tissue, in both electromechanical and vascular terms. Although various stem cells (including embryonic stem cells-ESC and induced pluripotent stem cells-iPSC) and autologous cells (skeletal myoblasts, mesenchymal stromal cells-MSC) have been using, the ideal cell source simply does not exist.[89] In addition, 3D bioprinting has the capacity to control the macro- and micro-architecture, and pore size/porosity of the scaffolds, but it is still challenging to vascularize thick tissue constructs.

3D BIOPRINTING OF HEART VALVES

There are four heart valves within the heart to ensure unidirectional flow of blood: the atrioventricular/inflow valves (mitral and tricuspid) and the semilunar/outflow valves (aortic and pulmonary). Each valve is composed of leaflets that are attached to a fibrous

annulus wall (root wall) and both leaflets and root wall are biomechanically and structurally anisotropic.[8,33] Leaflets and root walls mainly contain valve interstitial cells (VIC) and smooth muscle cells (SMC), respectively, with valvular endothelial cells (VEC) covered on the surface. The pathophysiology of valve disease is broad and calcific aortic valve disease (CAVD) is one of the most common valve abnormalities.[11] CAVD is most commonly treated with surgical or interventional repair or replacement at late stage and replacement options currently include mechanical or bioprosthetic valves.[43] Tissue engineering has great potential to address current limitations of non-living prosthetics by providing living constructs that can grow, remodel and integrate in the patients.

3D bioprinting, especially EBB, has been implemented to fabricate tissue engineered heart valve conduits. The advantages of using 3D bioprinting technique over traditional approaches are the ability to generate (1) anatomically accurate trileaflet valves; (2) mechanically heterogeneous valve conduits and (3) living engineered valves with spatial and temporal valve cells (VIC and SMC) distribution.

Currently, several valve models/designs have been used and reported for the printing. Duan *et al.* implemented a simple flat-shaped model and bioprinted trileaflet valve conduits using a combination of methacrylated hyaluronic acid and methacrylated gelatin with encapsulation of human aortic VIC (Figs. 3a and 3b).[18] Optimization of polymer ratio and concentration enabled control of the hydrogel viscosity and construct stiffness. The encapsulated cells were shown with high viability and matrix remodelling with sulfated GAG deposition (Fig. 3c). This simple testing model does not contain sinus (widenings between root wall and leaflets) and commissure (joined places between leaflets) structures which are very important to relieve abnormal stress and prevent blood back flow. Hockaday *et al.* used improved design with axially symmetric shape (Figs. 3d and 3e) and used a combination of 700 and 8000 MW poly(ethylene glycol) diacrylate (PEGDA) to print valve conduits with biomechanical heterogeneity, where the leaflets were more flexible, while the root remained relatively rigid.[37] The axially symmetric valve scaffolds were printed at various dimensions targeting at both paediatric and adult valve sizes (Fig. 3f). The same group also generated anatomically derived aortic valve geometric model using a μCT scan of a porcine aortic valve conduit freshly obtained at slaughter house (Fig. 3g).[36] The root and leaflet regions in the resulting scanned files were segmented *via* intensity thresholds (Figs. 3h–3j) and rendered into 3D geometries into separate stereolithography format of (STL) files.[36] The model maintained many anatomical features of native valves, like ostium and sinus. For heterogeneous bioprinting, the valve root (SMC laden hydrogel) was deposited first and subsequently, the leaflet region of the layer (VIC laden hydrogel) was extruded along its print paths (Fig. 3k).[16] These steps were repeated and the bioprinted aortic valve conduit with SMC and VIC encapsulated in the root and leaflet tissue, respectively, exhibited geometry comparable to the original image derived valve (Figs. 3l and 3m).[16]

Several studies have shown that the tissue engineered valve size and geometry play an important role in maintaining their functionality and stability under hemodynamic loading conditions.[2,82] Computational simulations showed inappropriate valve design may result in tissue compression in radial direction and eventually resulted into reduced leaflet size.[54] However, it is still unclear how the geometry influences hemodynamic properties and how cells in the bioprinted valve conduits respond to such changes and remodel the matrix. Bioprinting technique enables the fabrication of valve conduits with pre-designed size and geometry. It thus enables the determination of how size and geometry interact with hemodynamic stimulation to promote effective remodeling and cell phenotypes.

CHALLENGES AND FUTURE PERSPECTIVES

3D bioprinting stands as a promising technique for the development of cardiovascular tissues due to its ability to print heterogeneous and clinical relevant sized tissue constructs. Despite the great progress and promise, there are still many challenges that hinder its further applications and translations.

Need for High-Performance Bioinks and High-Resolution Bioprinter

Although many bioinks have been implemented, it is still a great challenge to develop ideal bioinks to bioprint biologically functional and mechanically robust tissue constructs. Ideal bioink should be (a) bioprintable (fast laser crosslinkable for laser based printing, extrudable for EBB), (b) support cardiovascular cell functions (adhesion, proliferation, differentiation, contraction *et al.*), (c) have comparable mechanical properties to native tissue/organ after crosslink, (d) be affordable and commercially available with appropriate regulatory guidelines for clinical use.[70] One of the major hurdles in currently available hydrogel based bioinks is to balance the cell functions and mechanical properties of crosslinked hydrogels. The high concentration of hydrogels results in high mechanical properties, low mobility and less spreading

of the encapsulated cells. One of the interesting directions in bioprinting is to develop *in situ* crosslinkable bioinks with spatially and temporally controllable crosslink rate and degree.[53,81] In addition, advanced biofabrication techniques are desirable to fabricate scaffold-free cell aggregation based bioinks in high throughput to decrease the fusion time and enhance mechanical properties and maturation.

Currently, a number of bioprinters, mainly extrusion based bioprinters, have been commercialized. The commercialization of other types of bioprinter is limited due to the limitation of bioink development. Ideally, the bioprinter should have resolution of submicron to bioprint matrix with an orientation which can induce the alignment of cardiovascular cells like cardiomyocytes and VIC. In addition, more cartridges should be used to implement more bioinks (i.e., matrix and cell types) for heterogeneous bioprinting. This is of significant important for whole heart bioprinting. Furthermore, more powerful and convenient software system is expected to control the printer in the customer defined way.

Combination with Other Biofabrication Techniques

To date bioprinting technology has not successfully printed any clinical relevant tissue constructs. One of the reasons is that each individual bioprinting technique has its own intrinsic disadvantages. Therefore, it makes more sense to combine two or multiple bioprinting techniques or combine bioprinting with other tissue engineering techniques. For example, the constructs or cell patterns generated by inkjet based bioprinting normally lack structural integrity and adequate mechanical properties for use *in vivo*. Xu *et al.* combined inkjet printing and electrospinning system to inkjet print rabbit elastic chondrocytes in a fibrin-collagen hydrogel on to electrospun polycaprolactone fibres forming a five-layer tissue construct.[97] Similarly, Visser *et al.*, in Malda group implemented melt electrospinning writing to reinforce soft hydrogels, forming highly organized high-porosity microfiber networks.[91] The bioprinting techniques also have great potentials to be combined with drug delivery strategy, cell sheet technique, nano/microfabrication techniques to extend the versatility and facilitate tissue/organ printing for translations and clinics.[64,98]

Bioprinting of Functional Cardiovascular Constructs

Currently, 3D bioprinting is still at its infancy stage and most of the applications/experiments, especially in cardiovascular areas. One of the major problems of the bioprinted constructs is a lack of mechanical strength and integrity due to the weak mechanical properties of hydrogel based bioinks.[86] The mechanical properties for most of bioprinted cardiovascular constructs, especially for blood vessel and myocardium, were not presented. For heart valve, the tensile and compressive properties of different bioprinted constructs were much smaller than the peak moduli of native valve tissues.[16,18] However, healthy valve cells are subjected to physiological strain range rather than failure strain. Within this range (~15% strain), valve cells can normally behave and function. The stiffness and moduli of bioinks were presented to be tunable and quite comparable to those of pulmonary valve leaflets in the physiological strain range. Although the hydrogel bioinks did not fulfill the full mechanical range of native valve tissue at the beginning, the tissue engineered constructs may be strengthened through further hemodynamic conditioning *via* collagen deposition and scaffold remodeling. Fully characterization of the mechanical properties and functions (like electrophysiological functions, hydrodynamic response, compliance, and remodeling) of the constructs are crucial to withstand the complex hemodynamic pressures and flows of the cardiac environment.[42,73] In addition to anatomical architecture and mechanical support, the bioprinted cardiovascular tissues should avoid thrombogenesis and resistant to calcification after implantation. This is of significant importance for *in vivo* and pre-clinical applications. This requires choosing and developing appropriate bioinks[17,105] It is also important to regulate the microenvironments, including matrix components, stiffness, and physiochemical stimuli, to control the differentiation of stem cells and other cell sources toward cardiovascular cell phenotypes.[15]

Bioprinting of Whole Heart

The bioprinted cardiac and valve tissue constructs are used as a patch or conduit for infarcted myocardium and valve tissue engineering, but they do not have an intact 3D structure with heart geometry.[92] The whole heart organ comprises of multiple cell types, ECM and multi-scale structures for pumping blood, and none of cardiovascular tissue has be bioprinted with full functions comparable to native tissue. The general structure of whole heart can be bioprinted. Hinton *et al.* recently used a thermoreversible support bath to enable freeform reversible embedding of suspended hydrogels bioprinting.[34] This process enables a resolution of ~200 μm and embryonic hearts printing with mechanical robustness and complex 3D internal and external anatomical architectures (Fig. 4). However, it is still very far away from our targets. One of the major hurdles is to generate multi-scale vascularization with high density and vascular tree-like net-

works. Again, this requires further improvement of both bioinks performances and bioprinter resolutions. In addition, prevascularization *in vitro* or *in vivo* can also promote formation of vascular network. Currently, miniature organs with partial functions can be considered as a future trend in organ printing and should be more practical strategy and be a transition toward fully functioning organs.

CONCLUSIONS

3D bioprinting has been gained enormous attentions as a fabrication technique for producing biological products, especially cardiovascular tissues. Various bioinks with multiple cells, biomaterials and biomolecules can be bioprinted using different printing mechanisms. Cardiovascular constructs, i.e., vascularized constructs, myocardium and heart valve conduits, have been successfully bioprinting with decent resolution, similar architecture to the native tissue and certain functions. However, this group of techniques is still in its infancy and many challenges remain for generating tissue/organ analogs with fully biological functions and complex microarchitecture. Therefore, more research efforts should be dedicated to develop more high-performance bioinks and high-resolution bioprinters. There is much promise that combining 3D bioprinting techniques with other tissue engineering, biofabrication and biological techniques will enable significant improvement for cardiovascular tissue engineering applications and further clinical uses.

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REFERENCES

[1]Ali, M., E. Pages, A. Ducom, A. Fontaine, and F. Guillemot. Controlling laser-induced jet formation for bioprinting mesenchymal stem cells with high viability and high resolution. *Biofabrication* 6:045001, 2014.

[2]Barannyk, O., and P. Oshkai. The influence of the aortic root geometry on flow characteristics of a prosthetic heart valve. *J. Biomech. Eng.* 137:051005, 2015.

[3]Bertassoni, L. E., J. C. Cardoso, V. Manoharan, A. L. Cristino, N. S. Bhise, W. A. Araujo, P. Zorlutuna, N. E. Vrana, A. M. Ghaemmaghami, M. R. Dokmeci, and A. Khademhosseini. Direct-write bioprinting of cell-laden methacrylated gelatin hydrogels. *Biofabrication* 6:024105, 2014.

[4]Bertassoni, L. E., M. Cecconi, V. Manoharan, M. Nikkhah, J. Hjortnaes, A. L. Cristino, G. Barabaschi, D. Demarchi, M. R. Dokmeci, Y. Z. Yang, and A. Khademhosseini. Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab Chip* 14:2202–2211, 2014.

[5]Billiet, T., E. Gevaert, T. De Schryver, M. Cornelissen, and P. Dubruel. The 3d printing of gelatin methacrylamide cell-laden tissue-engineered constructs with high cell viability. *Biomaterials* 35:49–62, 2014.

[6]Billiet, T., M. Vandenhaute, J. Schelfhout, S. Van Vlierberghe, and P. Dubruel. A review of trends and limitations in hydrogel-rapid prototyping for tissue engineering. *Biomaterials* 33:6020–6041, 2012.

[7]Bouten, C. V. C., P. Y. W. Dankers, A. Driessen-Mol, S. Pedron, A. M. A. Brizard, and F. P. T. Baaijens. Substrates for cardiovascular tissue engineering. *Adv. Drug Deliv. Rev.* 63:221–241, 2011.

[8]Butcher, J. T., G. J. Mahler, and L. A. Hockaday. Aortic valve disease and treatment: The need for naturally engineered solutions. *Adv. Drug Deliv. Rev.* 63:242–268, 2011.

[9]Catros, S., F. Guillemot, A. Nandakumar, S. Ziane, L. Moroni, P. Habibovic, C. van Blitterswijk, B. Rousseau, O. Chassande, J. Amedee, and J. C. Fricain. Layer-by-layer tissue microfabrication supports cell proliferation in vitro and in vivo. *Tissue Eng. C* 18:62–70, 2012.

[10]Chang, C. C., E. D. Boland, S. K. Williams, and J. B. Hoying. Direct-write bioprinting three-dimensional biohybrid systems for future regenerative therapies. *J. Biomed. Mater. Res. B* 98B:160–170, 2011.

[11]Chen, J. H., and C. A. Simmons. Cell-matrix interactions in the pathobiology of calcific aortic valve disease critical roles for matricellular, matricrine, and matrix mechanics cues. *Circ. Res.* 108:1510–1524, 2011.

[12]Cui, X. F., and T. Boland. Human microvasculature fabrication using thermal inkjet printing technology. *Biomaterials* 30:6221–6227, 2009.

[13]Cui, X. F., T. Boland, D. D. D'Lima, and M. K. Lotz. Thermal inkjet printing in tissue engineering and regenerative medicine. *Recent Pat. Drug Deliv. Formul.* 6:149–155, 2012.

[14]Derby, B. Printing and prototyping of tissues and scaffolds. *Science* 338:921–926, 2012.

[15]Duan, B., L. A. Hockaday, S. Das, C. Y. Xu, and J. T. Butcher. Comparison of mesenchymal stem cell source differentiation towards human pediatric aortic valve interstitial cells within 3d engineered matrices. *Tissue Eng. C* 21:795–807, 2015.

[16]Duan, B., L. A. Hockaday, K. H. Kang, and J. T. Butcher. 3D bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. *J. Biomed. Mater. Res., Part A* 101A:1255–1264, 2013.

[17]Duan, B., L. A. Hockaday, E. Kapetanovic, K. H. Kang, and J. T. Butcher. Stiffness and adhesivity control aortic valve interstitial cell behavior within hyaluronic acid based hydrogels. *Acta Biomater.* 9:7640–7650, 2013.

[18]Duan, B., E. Kapetanovic, L. A. Hockaday, and J. T. Butcher. Three-dimensional printed trileaflet valve conduits using biological hydrogels and human valve interstitial cells. *Acta Biomater.* 10:1836–1846, 2014.

[19]Duan, B., and M. Wang. Customized ca-p/phbv nanocomposite scaffolds for bone tissue engineering: design, fabrication, surface modification and sustained re-

lease of growth factor. *J. R. Soc. Interface* 7:S615–S629, 2010.
[20]Duan, B., and M. Wang. Selective laser sintering and its application in biomedical engineering. *MRS Bull.* 36:998–1005, 2011.
[21]Duan, B., M. Wang, W. Y. Zhou, W. L. Cheung, Z. Y. Li, and W. W. Lu. Three-dimensional nanocomposite scaffolds fabricated via selective laser sintering for bone tissue engineering. *Acta Biomater.* 6:4495–4505, 2010.
[22]Gaebel, R., N. Ma, J. Liu, J. J. Guan, L. Koch, C. Klopsch, M. Gruene, A. Toelk, W. W. Wang, and P. Mark. Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. *Biomaterials* 32:9218–9230, 2011.
[23]Gaetani, R., P. A. Doevendans, C. H. G. Metz, J. Alblas, E. Messina, A. Giacomello, and J. P. G. Sluijtera. Cardiac tissue engineering using tissue printing technology and human cardiac progenitor cells. *Biomaterials* 33:1782–1790, 2012.
[24]Gaetani, R., D. A. M. Feyen, V. Verhage, R. Slaats, E. Messina, K. L. Christman, A. Giacomello, P. A. F. M. Doevendans, and J. P. G. Sluijter. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. *Biomaterials* 61:339–348, 2015.
[25]Gao, Q., Y. He, J. Z. Fu, A. Liu, and L. Ma. Coaxial nozzle-assisted 3d bioprinting with built-in microchannels for nutrients delivery. *Biomaterials* 61:203–215, 2015.
[26]Gao, G. F., A. F. Schilling, K. Hubbell, T. Yonezawa, D. Truong, Y. Hong, G. H. Dai, and X. F. Cui. Improved properties of bone and cartilage tissue from 3D inkjet-bioprinted human mesenchymal stem cells by simultaneous deposition and photocrosslinking in peg-gelma. *Biotechnol. Lett.* 37:2349–2355, 2015.
[27]Gerstle, T. L., A. M. S. Ibrahim, P. S. Kim, B. T. Lee, and S. J. Lin. A plastic surgery application in evolution: Three-dimensional printing. *Plast. Reconstr. Surg.* 133:446–451, 2014.
[28]Go, A. S., D. Mozaffarian, V. L. Roger, E. J. Benjamin, J. D. Berry, M. J. Blaha, S. F. Dai, E. S. Ford, C. S. Fox, S. Franco, H. J. Fullerton, C. Gillespie, S. M. Hailpern, J. A. Heit, V. J. Howard, M. D. Huffman, S. E. Judd, B. M. Kissela, S. J. Kittner, D. T. Lackland, J. H. Lichtman, L. D. Lisabeth, R. H. Mackey, D. J. Magid, G. M. Marcus, A. Marelli, D. B. Matchar, D. K. McGuire, E. R. Mohler, C. S. Moy, M. E. Mussolino, R. W. Neumar, G. Nichol, D. K. Pandey, N. P. Paynter, M. J. Reeves, P. D. Sorlie, J. Stein, A. Towfighi, T. N. Turan, S. S. Virani, N. D. Wong, D. Woo, M. B. Turner, A. H. A. S. Comm, and S. S. Subcomm. Heart disease and stroke statistics-2014 update a report from the American heart association. *Circulation* 129:E28–E292, 2014.
[29]Gou, M. L., X. Qu, W. Zhu, M. L. Xiang, J. Yang, K. Zhang, Y. Q. Wei, and S. C. Chen. Bio-inspired detoxification using 3d-printed hydrogel nanocomposites. *Nat. Commun.* 5:3774, 2014.
[30]Guillemot, F., V. Mironov, and M. Nakamura. Bioprinting is coming of age: Report from the international conference on bioprinting and biofabrication in bordeaux (3b'09). *Biofabrication* 2:010201, 2010.
[31]Guillotin, B., A. Souquet, S. Catros, M. Duocastella, B. Pippenger, S. Bellance, R. Bareille, M. Remy, L. Bordenave, J. Amedee, and F. Guillemot. Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. *Biomaterials* 31:7250–7256, 2010.
[32]Haraguchi, Y., T. Shimizu, M. Yamato, and T. Okano. Concise review: cell therapy and tissue engineering for cardiovascular disease. *Stem Cells and Translational Medicine*. 1:136–141, 2012.
[33]Hasan, A., K. Ragaert, W. Swieszkowski, S. Selimovic, A. Paul, G. Camci-Unal, M. R. K. Mofrad, and A. Khademhosseini. Biomechanical properties of native and tissue engineered heart valve constructs. *J. Biomech.* 47:1949–1963, 2014.
[34]Hinton, T. J., Q. Jallerat, R. N. Palchesko, J. H. Park, M. S. Grodzicki, H. J. Shue, M. H. Ramadan, A. R. Hudson, and A. W. Feinberg. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Sci. Adv.* 1:e1500758, 2015.
[35]Hirt, M. N., A. Hansen, and T. Eschenhagen. Cardiac tissue engineering state of the art. *Circ. Res.* 114:354–367, 2014.
[36]Hockaday, L. A., B. Duan, K. H. Kang, and J. T. Butcher. 3D printed hydrogel technologies for tissue engineered heart valves. *3D Print. Addit. Manuf.* 1:122–136, 2014.
[37]Hockaday, L. A., K. H. Kang, N. W. Colangelo, P. Y. C. Cheung, B. Duan, E. Malone, J. Wu, L. N. Girardi, L. J. Bonassar, H. Lipson, C. C. Chu, and J. T. Butcher. Rapid 3D printing of anatomically accurate and mechanically heterogeneous aortic valve hydrogel scaffolds. *Biofabrication* 4:035005, 2012.
[38]Horch, R. E., U. Kneser, E. Polykandriotis, V. J. Schmidt, J. M. Sun, and A. Arkudas. Tissue engineering and regenerative medicine—where do we stand? *J. Cell Mol. Med.* 16:1157–1165, 2012.
[39]Horvath, L., Y. Umehara, C. Jud, F. Blank, A. Petri-Fink, and B. Rothen-Rutishauser. Engineering an in vitro air-blood barrier by 3D bioprinting. *Sci. Rep.* 5:7974, 2015.
[40]Hribar, K. C., P. Soman, J. Warner, P. Chung, and S. C. Chen. Light-assisted direct-write of 3D functional biomaterials. *Lab Chip* 14:268–275, 2014.
[41]Jakab, K., C. Norotte, F. Marga, K. Murphy, G. Vunjak-Novakovic, and G. Forgacs. Tissue engineering by self-assembly and bio-printing of living cells. *Biofabrication* 2:022001, 2010.
[42]Jana, S., and A. Lerman. Bioprinting a cardiac valve. *Biotechnol. Adv.* 33:1503–1521, 2015.
[43]Jana, S., B. J. Tefft, D. B. Spoon, and R. D. Simari. Scaffolds for tissue engineering of cardiac valves. *Acta Biomater.* 10:2877–2893, 2014.
[44]Jia, J., D. J. Richards, S. Pollard, Y. Tan, J. Rodriguez, R. P. Visconti, T. C. Trusk, M. J. Yost, H. Yao, R. R. Markwald, and Y. Mei. Engineering alginate as bioink for bioprinting. *Acta Biomater.* 10:4323–4331, 2014.
[45]Kang, K. H., L. A. Hockaday, and J. T. Butcher. Quantitative optimization of solid freeform deposition of aqueous hydrogels. *Biofabrication* 5:035001, 2013.
[46]Kirchmajer, D. M., R. Gorkin, and M. I. H. Panhuis. An overview of the suitability of hydrogel-forming polymers for extrusion-based 3d-printing. *J. Mater. Chem. B* 3:4105–4117, 2015.
[47]Kolesky, D. B., R. L. Truby, A. S. Gladman, T. A. Busbee, K. A. Homan, and J. A. Lewis. 3d bioprinting of vascularized, heterogeneous cell-laden tissue constructs. *Adv. Mater.* 26:3124–3130, 2014.
[48]Kucukgul, C., S. B. Ozler, I. Inci, E. Karakas, S. Irmak, D. Gozuacik, A. Taralp, and B. Koc. 3d bioprinting of biomimetic aortic vascular constructs with self-supporting cells. *Biotechnol. Bioeng.* 112:811–821, 2015.

[49]Lantada, A. D., and P. L. Morgado. Rapid prototyping for biomedical engineering: current capabilities and challenges. *Annu. Rev. Biomed. Eng*. 14:73–96, 2012.
[50]Lee, V. K., D. Y. Kim, H. G. Ngo, Y. Lee, L. Seo, S. S. Yoo, P. A. Vincent, and G. H. Dai. Creating perfused functional vascular channels using 3d bio-printing technology. *Biomaterials* 35:8092–8102, 2014.
[51]Lee, V. K., A. M. Lanzi, H. Ngo, S. S. Yoo, P. A. Vincent, and G. H. Dai. Generation of multi-scale vascular network system within 3d hydrogel using 3d bio-printing technology. *Cell. Mol. Bioeng*. 7:460–472, 2014.
[52]Levato, R., J. Visser, J. A. Planell, E. Engel, J. Malda, and M. A. Mateos-Timoneda. Biofabrication of tissue constructs by 3d bioprinting of cell-laden microcarriers. *Biofabrication* 6:035020, 2014.
[53]Li, C., A. Faulkner-Jones, A. R. Dun, J. Jin, P. Chen, Y. Z. Xing, Z. Q. Yang, Z. B. Li, W. M. Shu, D. S. Liu, and R. R. Duncan. Rapid formation of a supramolecular polypeptide-DNA hydrogel for in situ three-dimensional multilayer bioprinting. *Angew. Chem. Int. Ed*. 54:3957–3961, 2015.
[54]Loerakker, S., G. Argento, C. W. J. Oomens, and F. P. T. Baaijens. Effects of valve geometry and tissue anisotropy on the radial stretch and coaptation area of tissue-engineered heart valves. *J. Biomech*. 46:1792–1800, 2013.
[55]Loo, Y. H., A. Lakshmanan, M. Ni, L. L. Toh, S. Wang, and C. A. E. Hauser. Peptide bioink: self-assembling nanofibrous scaffolds for three-dimensional organotypic cultures. *Nano Lett*. 15:6919–6925, 2015.
[56]Lundberg, M. S. Cardiovascular tissue engineering research support at the national heart, lung, and blood institute. *Circ. Res*. 112:1097–1103, 2013.
[57]Markstedt, K., A. Mantas, I. Tournier, H. M. Avila, D. Hagg, and P. Gatenholm. 3D bioprinting human chondrocytes with nanocellulose-alginate bioink for cartilage tissue engineering applications. *Biomacromolecules* 16:1489–1496, 2015.
[58]Mehesz, A. N., J. Brown, Z. Hajdu, W. Beaver, J. V. L. da Silva, R. P. Visconti, R. R. Markwald, and V. Mironov. Scalable robotic biofabrication of tissue spheroids. *Biofabrication* 3:025002, 2011.
[59]Melchels, F. P. W., M. A. N. Domingos, T. J. Klein, J. Malda, P. J. Bartolo, and D. W. Hutmacher. Additive manufacturing of tissues and organs. *Prog. Polym. Sci*. 37:1079–1104, 2012.
[60]Melchels, F. P. W., J. Feijen, and D. W. Grijpma. A review on stereolithography and its applications in biomedical engineering. *Biomaterials* 31:6121–6130, 2010.
[61]Miller, J. S., K. R. Stevens, M. T. Yang, B. M. Baker, D. H. T. Nguyen, D. M. Cohen, E. Toro, A. A. Chen, P. A. Galie, X. Yu, R. Chaturvedi, S. N. Bhatia, and C. S. Chen. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat. Mater*. 11:768–774, 2012.
[62]Mironov, V., N. Reis, and B. Derby. Bioprinting: a beginning. *Tissue Eng*. 12:631–634, 2006.
[63]Mironov, V., R. P. Visconti, V. Kasyanov, G. Forgacs, C. J. Drake, and R. R. Markwald. Organ printing: tissue spheroids as building blocks. *Biomaterials* 30:2164–2174, 2009.
[64]Mosadegh, B., G. L. Xiong, S. Dunham, and J. K. Min. Current progress in 3d printing for cardiovascular tissue engineering. *Biomed. Mater*. 10:034002, 2015.
[65]Murphy, S. V., and A. Atala. 3d bioprinting of tissues and organs. *Nat. Biotechnol*. 32:773–785, 2014.
[66]Murry, C. E., R. W. Wiseman, S. M. Schwartz, and S. D. Hauschka. Skeletal myoblast transplantation for repair of myocardial necrosis. *J. Clin. Invest*. 98:2512–2523, 1996.
[67]Norotte, C., F. S. Marga, L. E. Niklason, and G. Forgacs. Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials* 30:5910–5917, 2009.
[68]Novosel, E. C., C. Kleinhans, and P. J. Kluger. Vascularization is the key challenge in tissue engineering. *Adv. Drug Deliv. Rev*. 63:300–311, 2011.
[69]Ozbolat, I. T. Bioprinting scale-up tissue and organ constructs for transplantation. *Trends Biotechnol*. 33:395–400, 2015.
[70]Ozbolat, I. T., and M. Hospodiuk. Current advances and future perspectives in extrusion-based bioprinting. *Biomaterials* 76:321–343, 2016.
[71]Ozbolat, I. T., and Y. Yu. Bioprinting toward organ fabrication: challenges and future trends. *IEEE Trans. Biomed. Eng*. 60:691–699, 2013.
[72]Park, J. Y., J. H. Shim, S. A. Choi, J. Jang, M. Kim, S. H. Lee, and D. W. Cho. 3d printing technology to control bmp-2 and vegf delivery spatially and temporally to promote large-volume bone regeneration. *J. Mater. Chem. B* 3:5415–5425, 2015.
[73]Parvin, N. S., M. C. Blaser, J. P. Santerre, C. A. Caldarone, and C. A. Simmons. Biomechanical conditioning of tissue engineered heart valves: too much of a good thing? *Adv. Drug Deliv. Rev*. 96:161–175, 2016.
[74]Pati, F., D. H. Ha, J. Jang, H. H. Han, J. W. Rhie, and D. W. Cho. Biomimetic 3d tissue printing for soft tissue regeneration. *Biomaterials* 62:164–175, 2015.
[75]Pati, F., J. Jang, D. H. Ha, S. W. Kim, J. W. Rhie, J. H. Shim, D. H. Kim, and D. W. Cho. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. *Nat. Commun*. 5:3935, 2014.
[76]Paulsen, S. J., and J. S. Miller. Tissue vascularization through 3D printing: will technology bring us flow? *Dev. Dyn*. 244:629–640, 2015.
[77]Peltola, S. M., F. P. W. Melchels, D. W. Grijpma, and M. Kellomaki. A review of rapid prototyping techniques for tissue engineering purposes. *Ann. Med*. 40:268–280, 2008.
[78]Poldervaart, M. T., H. Gremmels, K. van Deventer, J. O. Fledderus, F. C. Oner, M. C. Verhaar, W. J. A. Dhert, and J. Alblas. Prolonged presence of vegf promotes vascularization in 3d bioprinted scaffolds with defined architecture. *J. Controlled Release* 184:58–66, 2014.
[79]Reffelmann, T., and R. A. Kloner. Cellular cardiomyoplasty—cardiomyocytes, skeletal myoblasts, or stem cells for regenerating myocardium and treatment of heart failure? *Cardiovasc. Res*. 58:358–368, 2003.
[80]Rouwkema, J., N. C. Rivron, and C. A. van Blitterswijk. Vascularization in tissue engineering. *Trends Biotechnol*. 26:434–441, 2008.
[81]Rutz, A. L., K. E. Hyland, A. E. Jakus, W. R. Burghardt, and R. N. Shah. A multimaterial bioink method for 3D printing tunable, cell-compatible hydrogels. *Adv. Mater*. 27:1607–1614, 2015.
[82]Sanders, B., S. Loerakker, E. S. Fioretta, D. J. P. Bax, A. Driessen-Mol, S. P. Hoerstrup, and F. P. T. Baaijens. Improved geometry of decellularized tissue engineered heart valves to prevent leaflet retraction. *Ann. Biomed. Eng*. 2015. doi:10.1007/s10439-10015-11386-10434.
[83]Saunders, R. E., and B. Derby. Inkjet printing biomaterials for tissue engineering: bioprinting. *Int. Mater. Rev*. 59:430–448, 2014.

[84]Schiele, N. R., D. T. Corr, Y. Huang, N. A. Raof, Y. B. Xie, and D. B. Chrisey. Laser-based direct-write techniques for cell printing. *Biofabrication* 2:032001, 2010.
[85]Schmidt, C. E., and J. M. Baier. Acellular vascular tissues: Natural biomaterials for tissue repair and tissue engineering. *Biomaterials* 21:2215–2231, 2000.
[86]Seol, Y. J., H. W. Kang, S. J. Lee, A. Atala, and J. J. Yoo. Bioprinting technology and its applications. *Eur. J. Cardiothorac. Surg*. 46:342–348, 2014.
[87]Sodian, R., D. Schmauss, M. Markert, S. Weber, K. Nikolaou, S. Haeberle, F. Vogt, C. Vicol, T. Lueth, B. Reichart, and C. Schmitz. Three-dimensional printing creates models for surgical planning of aortic valve replacement after previous coronary bypass grafting. *Ann. Thorac. Surg*. 85:2105–2109, 2008.
[88]Spadaccio, C., M. Chello, M. Trombetta, A. Rainer, Y. Toyoda, and J. A. Genovese. Drug releasing systems in cardiovascular tissue engineering. *J. Cell Mol. Med*. 13:422–439, 2009.
[89]Sui, R. Q., X. B. Liao, X. M. Zhou, and Q. Tan. The current status of engineering myocardial tissue. *Stem Cell Rev. Rep*. 7:172–180, 2011.
[90]Sun, X., W. Altalhi, and S. S. Nunes. Vascularization strategies of engineered tissues and their application in cardiac regeneration. *Adv. Drug Deliv. Rev*. 2015. doi: 10.1016/j.addr.2015.1006.1001.
[91]Visser, J., F. P. W. Melchels, J. E. Jeon, E. M. van Bussel, L. S. Kimpton, H. M. Byrne, W. J. A. Dhert, P. D. Dalton, D. W. Hutmacher, and J. Malda. Reinforcement of hydrogels using three-dimensionally printed microfibres. *Nat. Commun*. 6:6933, 2015.
[92]Wang, F., and J. J. Guan. Cellular cardiomyoplasty and cardiac tissue engineering for myocardial therapy. *Adv. Drug Deliv. Rev*. 62:784–797, 2010.
[93]Williams, J. M., A. Adewunmi, R. M. Schek, C. L. Flanagan, P. H. Krebsbach, S. E. Feinberg, S. J. Hollister, and S. Das. Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. *Biomaterials* 26:4817–4827, 2005.
[94]Williams, S. K., J. S. Touroo, K. H. Church, and J. B. Hoying. Encapsulation of adipose stromal vascular fraction cells in alginate hydrogel spheroids using a direct-write three-dimensional printing system. *Biores. Open Access*. 2:448–454, 2013.
[95]Wong, N. D. Epidemiological studies of chd and the evolution of preventive cardiology. *Nat. Rev. Cardiol*. 11:276–289, 2014.
[96]Woodfield, T. B. F., J. Malda, J. de Wijn, F. Peters, J. Riesle, and C. A. van Blitterswijk. Design of porous scaffolds for cartilage tissue engineering using a three-dimensional fiber-deposition technique. *Biomaterials* 25:4149–4161, 2004.
[97]Xu, T., K. W. Binder, M. Z. Albanna, D. Dice, W. X. Zhao, J. J. Yoo, and A. Atala. Hybrid printing of mechanically and biologically improved constructs for cartilage tissue engineering applications. *Biofabrication*. 5:015001, 2013.
[98]Xu, Y. F., and X. H. Wang. Application of 3d biomimetic models in drug delivery and regenerative medicine. *Curr. Pharm. Des*. 21:1618–1626, 2015.
[99]Yang, S. F., K. F. Leong, Z. H. Du, and C. K. Chua. The design of scaffolds for use in tissue engineering. Part II. Rapid prototyping techniques. *Tissue Eng*. 8:1–11, 2002.
[100]Yeh, R. W., S. Sidney, M. Chandra, M. Sorel, J. V. Selby, and A. S. Go. Population trends in the incidence and outcomes of acute myocardial infarction. *N. Engl. J. Med*. 362:2155–2165, 2010.
[101]Yeong, W. Y., C. K. Chua, K. F. Leong, and M. Chandrasekaran. Rapid prototyping in tissue engineering: challenges and potential. *Trends Biotechnol*. 22:643–652, 2004.
[102]Yu, Y., Y. H. Zhang, and I. T. Ozbolat. A hybrid bioprinting approach for scale-up tissue fabrication. *J. Manuf. Sci. Eng. Trans. ASME*. 136:61013, 2014.
[103]Zein, I., D. W. Hutmacher, K. C. Tan, and S. H. Teoh. Fused deposition modeling of novel scaffold architectures for tissue engineering applications. *Biomaterials* 23:1169–1185, 2002.
[104]Zhang, A. P., X. Qu, P. Soman, K. C. Hribar, J. W. Lee, S. C. Chen, and S. L. He. Rapid fabrication of complex 3D extracellular microenvironments by dynamic optical projection stereolithography. *Advanced Materials*. 24:4266–4270, 2012.
[105]Zheng, Y., J. M. Chen, M. Craven, N. W. Choi, S. Totorica, A. Diaz-Santana, P. Kermani, B. Hempstead, C. Fischbach-Teschl, J. A. Lopez, and A. D. Stroock. In vitro microvessels for the study of angiogenesis and thrombosis. *Proc. Natl Acad. Sci. USA* 109:9342–9347, 2012.