3D Bioprinting of cardiac tissue and cardiac stem cell therapy

MATTHEW ALONZO, SHWETA ANILKUMAR[1], BRIAN ROMAN[1], NISHAT TASNIM, and BINATA JODDAR

EL PASO, TEXAS

Cardiovascular tissue engineering endeavors to repair or regenerate damaged or ineffective blood vessels, heart valves, and cardiac muscle. Current strategies that aim to accomplish such a feat include the differentiation of multipotent or pluripotent stem cells on appropriately designed biomaterial scaffolds that promote the development of mature and functional cardiac tissue. The advent of additive manufacturing 3D bioprinting technology further advances the field by allowing heterogenous cell types, biomaterials, and signaling factors to be deposited in precisely organized geometries similar to those found in their native counterparts. Bioprinting techniques to fabricate cardiac tissue in vitro include extrusion, inkjet, laser-assisted, and stereolithography with bioinks that are either synthetic or naturally-derived. The article further discusses the current practices for postfabrication conditioning of 3D engineered constructs for effective tissue development and stability, then concludes with prospective points of interest for engineering cardiac tissues in vitro. Cardiovascular three-dimensional bioprinting has the potential to be translated into the clinical setting and can further serve to model and understand biological principles that are at the root of cardiovascular disease in the laboratory. (Translational Research 2019; 000:1–20)

Abbreviations: BMSCs = bone marrow-derived mesenchymal stem cells; CFs = cardiac fibroblasts; CMs = cardiomyocytes; CMR = cardiac magnetic resonance; CNT = carbon nanotube; CVD = cardiovascular disease; CSCs = cardiac stem cells; CT = computer tomography; EBB = extrusion-based bioprinting; ECs = endothelial cells; ECM = extracellular matrix; GelM2A = gelatin methacrylate; human iPSCs = human induced pluripotent stem cells; HUVECs = human umbilical vein endothelial cells; LAB = laser-assisted bioprinting; LIFT = laser induced forward transfer; MSCs = mesenchymal stem cells; PEGDA = poly(ethylene glycol) dimethacrylate; RGD = arginine-glycine-aspartate

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in most developed countries such as the United States and is a broad term given to a set of pathologies that affect the myocardium, heart valves, or vasculature in the body.[1,2] The progression of CVD usually leads to the deterioration of one or more of the structures and cells of the heart and will, at their end-stage, need replacement in order to improve the prognosis of patients affected. Current medical practices usually involve grafting tissues from the patient's own body, donors, animals, or synthetically

[1]Denotes equal contribution.

From the Inspired Materials & Stem-Cell Based Tissue Engineering Laboratory (IMSTEL), Department of Metallurgical, Materials and Biomedical Engineering, University of Texas at El Paso, El Paso, Texas; Border Biomedical Research Center, University of Texas at El Paso, El Paso, Texas.

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Reprint requests: Binata Joddar, Department of Metallurgical, Materials and Biomedical Engineering, University of Texas at El Paso, 500 W University Avenue, M201 J Engineering Building, El Paso, Texas. e-mail: bjoddar@utep.edu.

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made constructs. Autografts, such as coronary artery bypass grafts, are performed by harvesting part of the patient's own saphenous vein or other vessel to treat ischemia.[3] End stage heart failure is treated by allografting a heart from a donor,[4] while some valve replacement surgeries normally involve xenografting bovine or porcine heart valves.[5] Synthetic valves[5] and vascular grafts[6] can also be implanted to treat CVD. Although each type of graft holds promise in treating one of the pathologies associated with CVD, each has their set of disadvantages that include, but are not limited to, a shortage of donor organs that are readily available,[7] anticoagulation therapy,[8] immune rejection,[9] and limited durability.[10] As such, other avenues for readily available and compatible treatments are needed.

Cardiovascular tissue engineering endeavors to repair damaged or ineffective blood vessels, heart valves, and cardiac muscle.[11] Current strategies to accomplish such a feat include the differentiation of stem cells into mature and functional tissues on biomaterials that support the tissue's growth and development. The biomaterials of choice usually involve either natural or synthetic hydrogels, or decellularized matrices as they provide a porous, interconnected polymeric network that allow cells to migrate, proliferate, and receive the nutrients that are essential to their survival.[12] Moreover, because of their potential to reduce the immune rejection of grafts, decrease thrombogenic effects, and prospectively have tissues available on demand, the use of autologous and allogenic stem cells are a hot topic in cardiac tissue engineering.[13,14] Generation of cardiac structures requires the integration of cardiac fibroblasts, cardiomyocytes, and endothelial cells, derived from multiple stem cell sources (Fig 1).

Three-dimensional bioprinting technology, an additive manufacturing technique that employs a layer-by-layer approach, has been implemented to develop the next generation of cardiac patches. Numerous efforts have been made to 3D bioprint functional cardiac tissues-on-a-chip using biomaterials such as scaffolds or bioinks that could restore the functions of the damaged myocardium. Some of the biomaterials that were utilized to 3D bioprint myocardial tissue include alginate,[16] collagen,[17,18] gelatin,[19,20] hyaluronic acid,[21] and decellularized extracellular matrix scaffolds, among others.[22] Despite the many successes of 3D printed scaffold-based cardiac patches, they are not without imperfections. Scaffolds have a high probability of rapid degeneration, eventually resulting in reduced physical or mechanical stability.[23,24] However, 3D bioprinting of scaffold-free

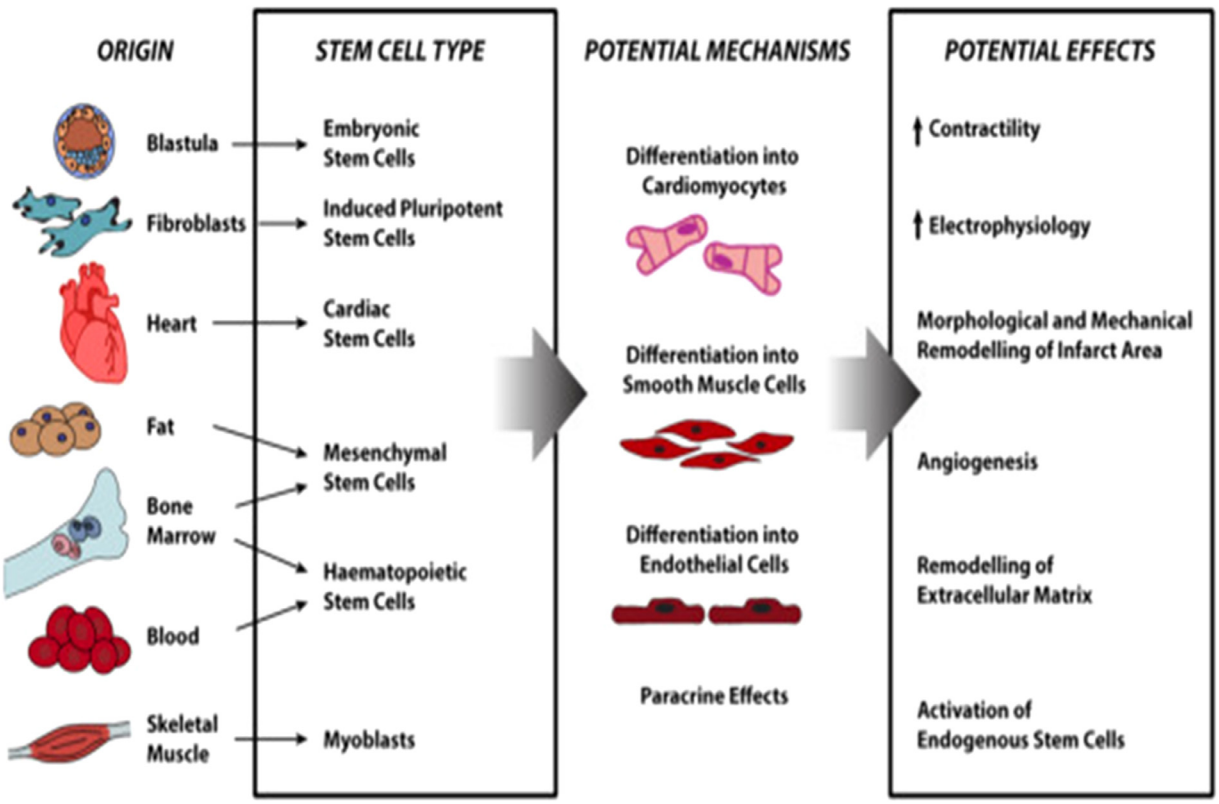

Fig 1. Cells for cardiac tissue engineering. These cells can be derived from multiple stem cell sources as shown in the figure. Reproduced with permission from Ref.[15]

cardiac patches have yielded satisfactory outcomes. Atmanli et al demonstrated the fabrication of functional cardiac patches using microcontact 3D bioprinting of double transgenic murine committed ventricular progenitors.[23] These patches were found to maintain the unique architecture of the native myocardial tissue.[23] A separate study conducted by Ong et al fabricated spontaneously beating biomaterial-free cardiac patches by 3D bioprinting mixed cell spheroids containing aggregates of human-induced pluripotent stem cell-derived cardiomyocytes, fibroblasts, and ECs.[24] Bioprinted cardiac patches fabricated from gelatin methacrylate-cardiac extracellular matrix (GelMA-cECM) hydrogel-based bioinks laden with human cardiac progenitor cells were found to increase the angiogenic potential and showed vascularization when applied on rat hearts.[25] Other research groups have also met with success in constructing scaffold-free 3D bioprinted cardiac patches exhibiting viability, vascularization, and engraftment of cells following implantation.[26,27] Motivated by these outcomes, we added fibrin to our previously optimized photopolymerizable gelatin-based bioink to fabricate cardiac cell-laden constructs with human-induced pluripotent stem cell-derived cardiomyocytes or CM cell lines and cardiac fibroblasts (CFs). The cell-laden bioprinted constructs were crosslinked via a two-step procedure including the visible-light crosslinking of furfuryl-gelatin followed by the chemical crosslinking of fibrinogen via thrombin and calcium chloride. The printed constructs retained stability for at least 5 days, and scanning electron microscopy imaging exposed an extremely porous, networked structure. Cells printed within the sheet structure showed excellent viability, proliferation, and expression of the Troponin-I cardiac marker. Furthermore, this 3D bioprinted cardiac patch promoted coupling between CMs and CFs, which forms the basis of normal physiology of the cardiac wall in vivo. This improved "cardiac patch" may be used as an advanced platform for drug cytotoxicity screening or unraveling triggers for heart diseases in vitro.[20] To summarize, 3D bioprinting technology can be effectively harnessed for the construction of cardiac patches that can be used to repair or replace diseased cardiac tissues and restore cardiac functionality to some extent. The article will further elaborate on 3D bioprinting techniques and bioinks that are extensively used in applications relating to tissue engineering and regenerative medicine, with a brief description on pre- and post-fabrication of 3D printed cardiac constructs.

PREFABRICATION OF 3D BIOPRINTED CONSTRUCTS

3D bioprinting of diseased or damaged cardiac tissue is preceded by the creation of a patient-specific 3D model that can either begin with clinical imaging or the implementation of computer-aided design models.[28] Medical image data can be used to computer-generate anatomically accurate models. The current imaging techniques to develop biologically relevant three-dimensional models include: electrocardiography-gated computer tomography (CT), volumetric 3D echocardiography, and cardiac magnetic resonance (CMR) as the imaging dataset needs to be volumetric.[29,30] Owing to its availability in abundance, low cost and lack of ionizing radiation, 3D echocardiography is a very appealing data source. A 3D transesophageal echocardiography data source is a better option to generate models of clearly imaged cardiac structures like ventricular chambers or valve leaflets.[31] However, ultrasound imaging is associated with artifact and unique limitations that can result in anatomic data loss within an ultrasound shadow, making CT, and CMR the most viable imaging techniques for 3D printing.[31] CT provides excellent spatial resolution[31] in addition to being able to image patients wearing pacemakers and metal implants which are not compatible with CMR scanning.[32] On the contrary, CMR can obtain high-resolution images without ionizing radiation, and can discern the tissue composition without the iodinated contrast media.[33] CMR images have been widely used to develop 3D printed models of congenital heart chambers and vasculature as well as for the reconstructive modeling of intracardiac tumors.[31,34]

The 3D anatomic information received from the CT, CMR, or 3D echocardiography volumetric imaging datasets is converted into a 3D patient-specific digital model of the cardiovascular tissue in a process known as "image segmentation." The evolution of the segmentation techniques can be attributed to an increasing fascination for anatomic modeling and a pressing need for personalized structural heart interventions. Earlier, segmentation was only based on CT images,[35-37] but recent studies have also included CMR images to replicate congenital heart and systemic vasculature disorders.[38-40] Researchers have also demonstrated the feasibility of reconstructing mitral leaflets and annulus from 3D transesophageal echocardiogram images and echocardiographic data acquired from multiple views or echocardiographic data combined with CT data.[41-43] Initially, the acquired image data set, from CT/MRI cardiac studies, is exported into a Digital Imaging and Communication in Medicine format, from which the target anatomic geometry is identified and segmented based on the threshold intensity of pixels in the grey-scale 2D image projections, axial, sagittal, and coronal, respectively.[44-46] The next step involves the creation of segmentation masks, where the software "stacks" individual 2D images of the heart tissue in such a manner

so as to group pixels that fall within the same intensity range and assign them to be printed with a single material.[44] These masks are then converted into the final patient-specific 3D digital cardiac models using rendering techniques and are saved as Standard Tessellation Language (.stl) files within a computer aided software, where they can be subjected to alterations and adjustments to make them more user-specific and finally, exported for 3D bioprinting (Fig 2).[47,48]

VARIOUS MODES OF BIOPRINTING

The different types of bioprinting processes are laser-based, droplet-based (inkjet, EHD-jet, acoustic, microvalve-based bioprinting), extrusion-based and stereolithography bioprinting to name a few. These are discussed in detail below.

Extrusion. Extrusion-based bioprinting is characterized by the expulsion of viscoelastic biomaterials called bioinks in cylindrical filaments to form 3D constructs in a layer-by-layer fashion.[49] The nozzle that dispenses the bioink has the ability to move in the x, y, and z directions and the printer may have a stage that can be adjusted. Pressure can be applied using 3 mechanisms to extrude the bioink, which may be laden with cells. The pneumatic-based approach drives utilizing pressurized air at a controlled volume flow rate while piston- and screw-based techniques mechanically force the biomaterials out of the nozzle. The downside to this technique is the need for pressures that sometimes tend to lyse cells. Increasing the diameter of the nozzle or utilizing viscous materials can dispense thicker filaments, which produce mechanically stable structures. Overall, the technique is slow, of moderate cost, produces low resolution, and requires specific matching of the material utilized with the implanting matrix to preserve viability.

Extrusion-based bioprinting with a cell-laden modified alginate bioink was implemented by Gaetani et al to produce constructs with human fetal CM progenitor cells. Their proof of concept study concluded that human fetal CM progenitor cells had high cell viability, retained their commitment for the cardiac lineage and expressed early cardiac transcription factors and the sarcomeric protein TroponinT within the 3D environment. The bioprinted cells were also able to migrate from the constructs and formed tube-like structure on the matrigel layer.[2] Zhang et al were able to assemble vascularized cardiac tissue using extrusion-based bioprinting. Using a composite bioink that was crosslinked through a two-step process and by introducing a gradient of nutrients radially within the printed filaments, ECs were able to migrate and form confluent tubular-like structures that resemble blood vessels. While they used neonatal rat CMs as a model cell type, such a system has the potential to be translated to human cardiac organoids through the application of human induced pluripotent stem cell-derived cardiomyocytes.[50]

Kumar et al used extrusion bioprinting to fabricate mono- and bilayered constructs, seeded with mouse mesenchymal stem cells (MSCs) in the former and

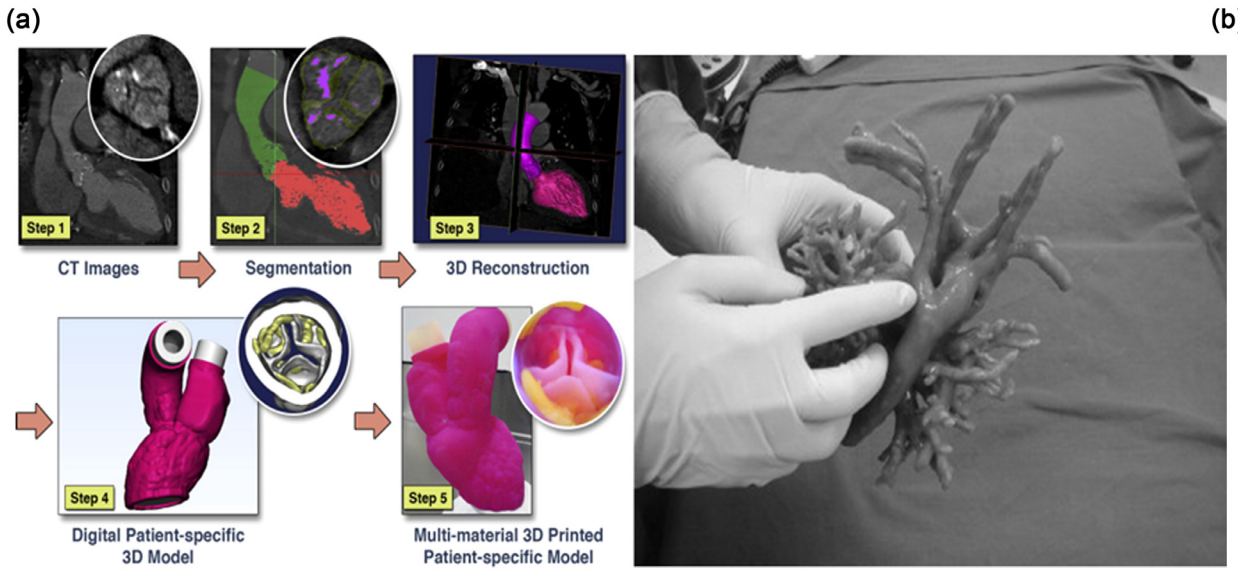

Fig 2. (a) Steps involved in the prefabrication of 3D printed patient-specific models.[31] (b) A 3D printed luminal replica of an aberrant retroesophageal left subclavian artery and right aortic arch for intraoperative use as congenital defect model.[39]

C2C12 cardiac myoblasts and Sandos inbred mouse (SIM)-derived 6-thioguanine- and ouabain-resistant (STO) fibroblasts in the latter (Fig 3).[18] The results of the study showed that the cells retained their viability and functionality in both cases after 5 days of culture showing that gelatin could be potentially used for the fabrication of cardiac tissues.

Inkjet. The roots of the 3D bioprinting technology can be traced back to ink jet printers, which were initially used in offices and later with personal computers in the

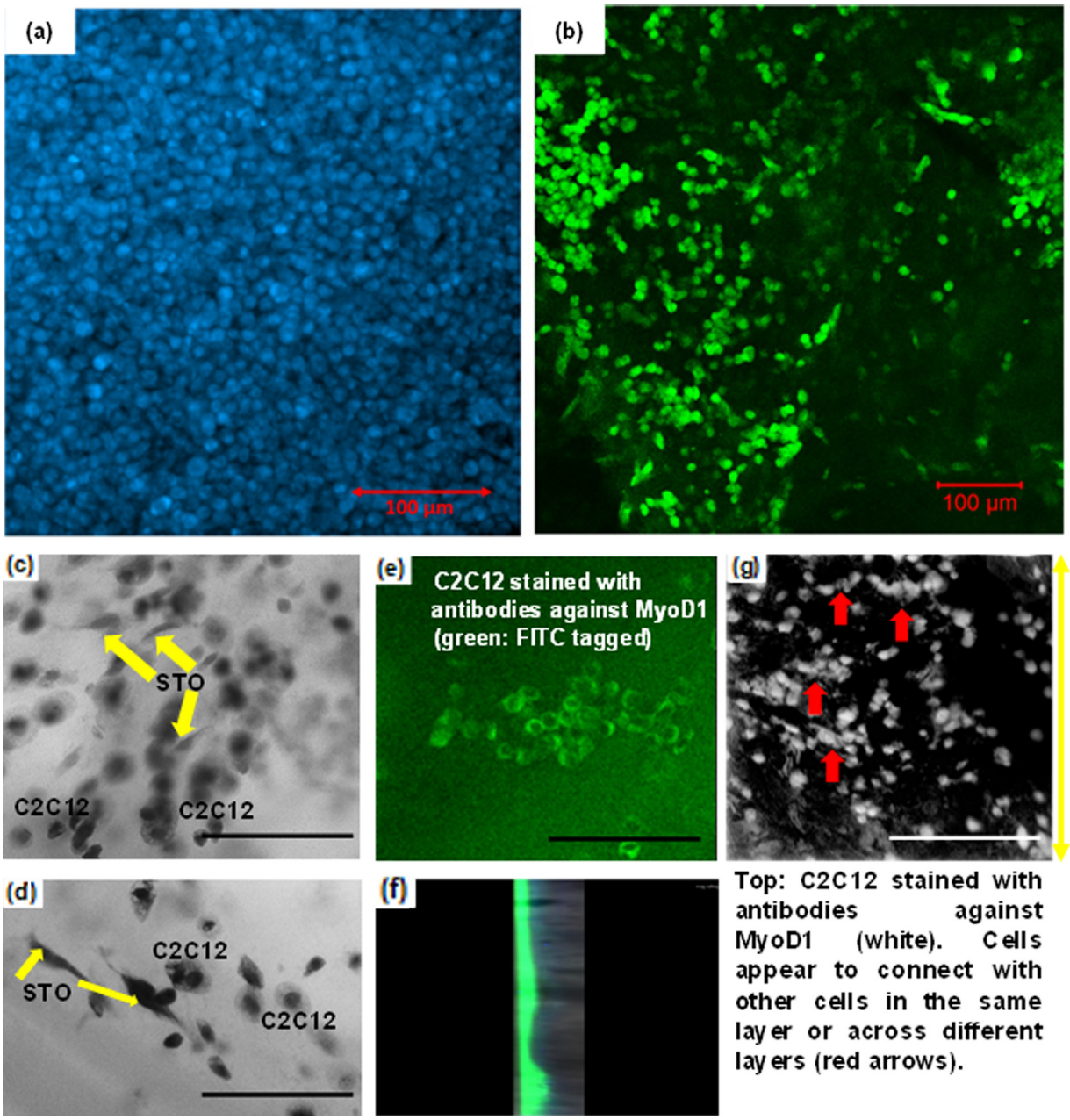

Fig 3. (a) Illustrates the viability of mouse MSCs stained with Hoechst (blue), 24 hour after printing (b) shows the retention of mouse MSC, prestained with PKH67 (green), within the bioprinted construct after 5 days of culture. In (c) and (d), shown are bright field z−scans of STO fibroblasts (elongated spindle shaped) co-cultured with C2C12 myoblasts cells [rounded enlarged, confirmed in (e) and in (g)]. Scale bar is 150 μm in (c), (d) and 200 μm in (e) and (g). In (e), a single plane (cross section) was imaged whereas in (g), a Z−scan was run spanning several planes as indicated with the arrow (right hand side). In (f), a single slice of z−stack section showing top layer (fluorescent: green for C2C12) and bottom layer (non-fluorescent: STO) is shown. Reproduced with permission from Ref.[19] (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

1980s. This technique works by depositing droplets of ink through narrow orifices onto a piece of paper. In the early 2000s, Cui et al transformed the traditional ink jet printer into a bioprinter by replacing the ink in the cartridges with a "bioink" containing cells.[51] The inkjet printer is capable of depositing cell-encapsulated droplets of sizes of 1−100 picoliters[52] with densities as high as 10,000−30,000 cells/drop[53] through a noncontact nozzle. Among the different types of inkjet-based printers, thermal and piezoelectric drop-on demand printers continue to be the most commonly used for bioprinting. The thermal inkjet system comprises of an ink chamber with several small nozzles and a heating element. The application of a short current pulse to the heating element raises the temperature of the ink surrounding the heating element resulting in the formation of a bubble that coerces the ink out of the nozzle orifice.[54] On the contrary, the piezoelectric inkjet printer uses piezoelectric crystals, which are situated, at the back of the ink chamber. These crystals start vibrating when an electric charge is applied to them. These inward vibrations force a small amount of ink out of the nozzle.[55] Inkjet printers can generate high resolution structures (20−100 μm) at the deposition rate of 1−1000 drops/s.[56] Boland et al successfully demonstrated that a high level of control could be exercised over the cell dispensing while simultaneously maintaining remarkable viability and functionality of the different cell types that were used.[57-59] However, inkjet printers are only compatible with low-viscosity bioinks (<10 cP) owing to the dispensing mechanisms and noncontact nature of the inkjet printer, resulting in structures with weaker mechanical properties as compared to other printers.[60,61]

Biomaterials like hydrogels, fibrin, agar, alginate, and collagen have been used with inkjet printers in previous studies.[62] Despite its success in tissue engineering, the inkjet aided bioprinting of the cardiac tissue specifically is still in its infancy stage. Xu et al used the inkjet printing technique to develop a hierarchical design of functional cardiac pseudo tissues with a beating cell response, balanced by porosity for mass and structural support.[63] Implementation of a modified inkjet printer created 3D rectangular cell sheets with alternating layers of cell-laden hydrogels. The microstructures of the construct were crosslinked by spraying microdroplets of $CaCl_2$ into an alginate hydrogel precursor solution containing primary feline adult CMs. The results of the study indicated that the cells adhered well to the alginate scaffold and also mimicked the native cardiac ECM. When subjected to mild electrical stimuli, these engineered tissues were also found to exhibit contractile behavior.[63] Further studies demonstrated that precise cell placement could significantly improve the production of cardiac tissues with off-the-shelf inkjet technology.[64] The feasibility of incorporating vascularization into the printed cardiac patches using the inkjet technology was also demonstrated by Cui et al, by developing micron-sized fibrin channels with the human vascular endothelial cells used in the study, aligning themselves inside the channels, and forming confluent linings, mimicking blood vessels within the printed structures as depicted in Fig 4.[61]

Laser-assisted. Laser-assisted bioprinting (LAB) has been shown to facilitate the formation of in vitro tissue constructs with properties including high resolution, high printing speed, large cell densities, and an increased viability of cells. The foremost method encompassing laser-assisted technology is that of laser-induced forward transfer (LIFT). This method involves the use of 2 parallel slides, namely a donor (upper) and a collector (lower) slide, to deliver the desired tissue structure. Beneath the donor slide lies a laser-absorbing metal, such as gold, titanium, or silver, covered by the biomaterial to be transferred.[65] As laser pulses are absorbed by the metal, this one evaporates, causing the biomaterial to fall onto the collector slide, with laser energies having been shown to range from 65 nJ to 190 μJ.[65-67] Bioinks associated to the use of LIFT include combinations of alginate, glycerol, and ethylenediaminetetraacetic acid, but studies have shown this LAB technique to be capable of forming tissue constructs using biomaterials of viscosities ranging from 1 to 400 mPa s.[68,69] In addition, due to its high droplet selectivity, LIFT is able provide both 2D and 3D models by stacking biomaterial droplets as necessary.[65,70] A study by Duocastella, formally demonstrates the spatial resolution of LIFT at varying film to substrate distances and at different set laser pulse energies.[66] This analysis of a LIFT system demonstrates LAB's ability to produce an array of droplets with constant size and shape, portraying the consistent pulse of ink required for reliable bioprinting. The printing speed has also been explored; with velocities, varying based on the bioink of choice and separated into a fast and a slow jet stream. The fast jet is propelled first, reaching speeds up to 150 m/s, followed by a second jet that materializes after a certain laser fluence is reached and settles at about 4.6 m/s for single layer transfer.[5] Such speeds are important for the survival of cardiac cells during bioprinting as printing speeds higher than 200 m/s can compromise viability.[67,69] This LAB feature permits cardiac cell survival to reach a minimum of about 90% and a maximum of up to 100% postbioprinting, maintaining a majority of the cells' functions and morphology.[70,71] In addition to a high cell viability, LAB is capable of providing a cell density of 10^8 cells/mL.[5] Furthermore, LAB has been found capable of selecting a single cell for transfer.[3] Because of such attributes, LABs are not commercially

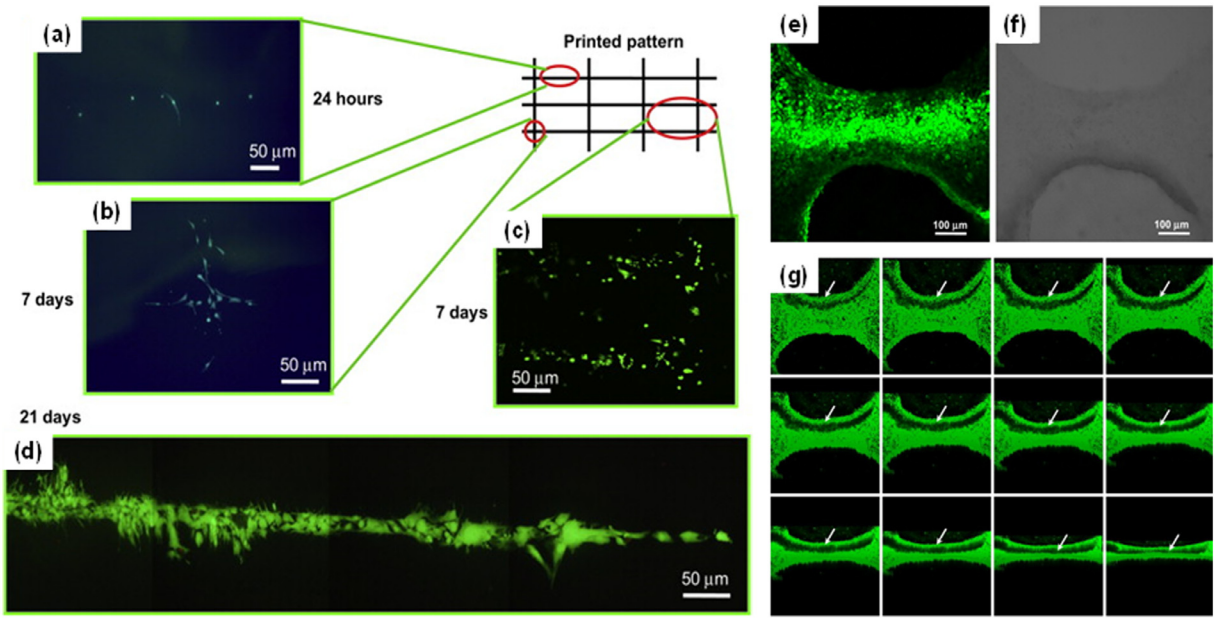

Fig 4. Human vasculature printed using a modified thermal inkjet printer. Samples indicating different positions of the printed grid pattern showing the proliferation of HVECs after (a) 24 hour of culture (b and c) 7 days of culture and (d) 21 days of culture. The cells were able to network and proliferate to form a confluent lining. Fig 4(e) shows the fluorescent stained printed cells that are aligned within the fibrin scaffold; (f) DIC image of the fibrin scaffold; (g) Confocal series of images showing the proliferated HMVEC sealed inside of the fibrin channel and the formation of a distinct tubular structure. Reproduced with permission from Ref.[61]

available, and their cost is high with units reaching up to U.S. $200,000 as formally reviewed by Ozbolat et al.[72]

The multiple characteristics of LAB equip it with a number of functions in cardiac tissue engineering. One application is the printing of endothelial colony-forming cells and adipose-derived stem cells in a 3D manner. By providing a third dimension, one is able to observe cell-to-cell and cell-to-environment interactions and their effect on cellular morphology as would be observed within cardiac ECM.[73] The addition of a cell-to-cell network is imperative as in vitro studies have shown these to cause the formation of newly developed blood vessels.[74] The use of LAB has been further explored in the printing of different cell constructs adjacent to each other.[5] This is possible due to LAB's high resolution, enabling the printing of complex tissues containing multiple cells such as that of the myocardium. The technical complexity surrounding the heart led Gaebel et al to use LAB's high resolution on a damaged heart by placing human umbilical vein endothelial cells (HUVECs) next to MSCs (Fig 5). This led to the discovery that MSCs cause a decrease in collagen formation and an increase in capillary density when placed on an injured heart.[8] Along with LAB's high resolution, clogging and contamination are eliminated as these bioprinters lack an orifice as those of extrusion and ink-jet types. Additional bioprinting benefits include control over the number of cells per droplet and a high viability of cardiac cells. This allows for the printing of single cardiac cells within a biomaterial with a great majority of the cells preserving their morphology and function. Nonetheless, drawbacks still accompany this system, as it is not yet sold commercially and is done so at a very high price. LAB's resolution is also dependent on many parameters to acquire the desired resolution including bioink viscosity, layer thickness of the bioink, and laser influence.[5]

Stereolithography. Stereolithography (SLA) is a laser-assisted bioprinting system which creates 3D structures by photocuring of photopolymerizable liquid polymers resulting in more realistic microstructures compared to other techniques.[76] Photocuring or photopolymerization is a process in which the liquid polymer is cross-linked by exposing the predesigned patterns using a laser beam of UV, infrared, or visible light.[77] This bioprinting system integrates a light source, a liquid photopolymer reservoir, and a three-axis motion stage.[77] Exposure to the laser beam photocures the pattern over the path traced on the liquid polymers and joins it to the layer below, allowing a layer-by-layer printing to form a 3D structure. Depending on the selection of laser sources SLA can have single- or two-photon absorption. Two-photon SLA has higher printing resolution than single-photon, because in two-photon absorption photopolymerization is induced in a very precise region.[76,78]

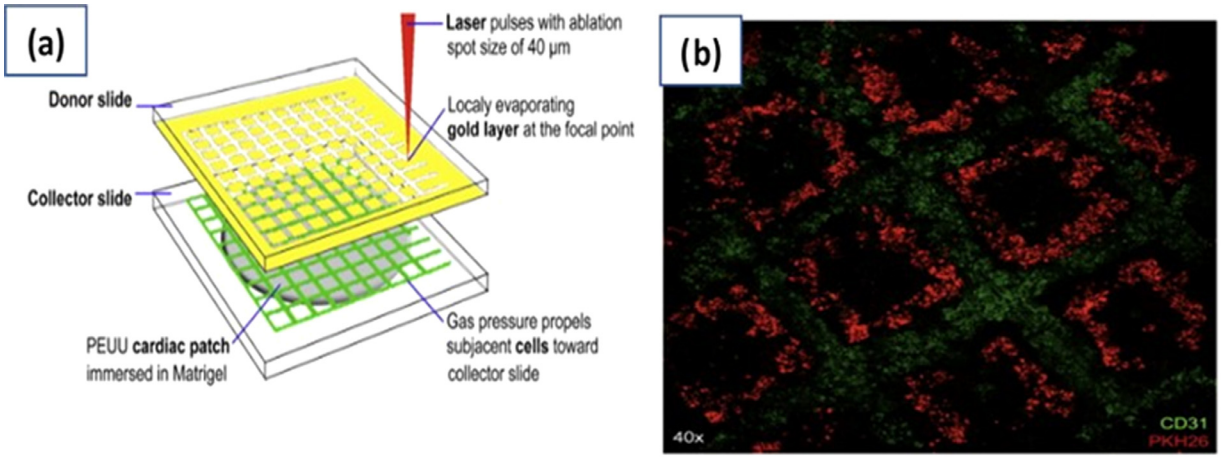

Fig 5. Bioprinting using laser induced forward transfer (LIFT). (a) LIFT diagram; (b) HUVECs were printed in a grid arrangement with hMSCs being printed in a square arrangement within the HUVEC grid lines. Reproduced with permission from Ref.[75]

In cardiovascular surgery, heart valve and vascular replacement are often required. The fabrication of heart valve scaffolds using SLA technique has been investigated for more than a decade. The tissue-engineered therapeutic approach for aortic stenosis needs the fabrication of aortic valve which would provide higher orifice contraction and better hemodynamic impact.[79] A study was performed on 3D stenotic aortic valve creating 3 different shapes of valves by SLA printing and their hemodynamic effect was observed by 3D echocardiography.[79] This study provided important information in determining the impact of aortic stenosis on patient hemodynamics and the relation among tissue structures, blood flow, and pressure in the heart.[79] In addition, researchers used SLA technique to generate biocompatible and biodegradable heart valve scaffolds using biopolymers, such as poly-4-hydroxybutyrate (P4HB) and a polyhydroxyoctanoate (PHOH).[80] They fabricated the complex anatomic structure of a human pulmonary and aortic homograft in vitro and in vivo from a stereolithographic model without the need for suturing. The heart valves opened and closed synchronously under subnormal and supranormal flow and pressure conditions, while analyzed in a pulsatile bioreactor.[80]

In the field of cardiac stem cell therapy, MSCs loaded in hydrogel patches have been used to develop a therapeutic approach for myocardial infarction.[81] The MSC-laden, multiple microchanneled hydrogel patches were fabricated using SLA printing technique with controlled diameters (500 μm). The researchers used BMSCs and poly(ethylene glycol) dimethacrylate to construct the cell-laden patches.[81] The microchannels serving as drainage of cell-secreted growth factors, were fabricated using SLA assembly unit. Echocardiography showed improvement of cardiac function with reduced cardiac remodeling after the cell-gel microchanneled construct was implanted in the damaged heart of a rat.[81] The microchanneled gel patch provided a better cardiac function with directed angiogenic potential in a reproducible manner compared to the microchannel-free gel patch, which was used as a control. The researchers proposed that using micro-SLA would further decrease the diameter (eg, 200−300 μm) of the microchannels providing improved cell-secreting factors into implanted tissue.[81]

SLA technique in bioprinting 3D cardiac patches and heart valves using stem cells and polymeric bioinks has great promise in cardiovascular tissue engineering (Fig 6). The major advantages of SLA in this field would be reduced printing time, a high degree of fabrication accuracy, and high cell viability due to its nozzle-free technique.[82] In contrast, fabricating 3D cardiac tissue scaffold using SLA can have the adverse effects of using the lasers and optics needed for the photocuring purposes, which are expensive and also affect the cell viability.[76,83] However, this disadvantage can be avoided by using visible light, such as lithium phenyl-2,4,6-trimethylbenzoylphosphinate,[84] camphorquinone[85] and eosin Y.[82] SLA-based bioprinting has imparted printing time to be only around 30 minutes[86] and printing resolution to be 200 μm.[84] Recently, engineers have developed a new SLA process of 4 cell-based inks which provides even higher printing speed, better resolution, and multimaterial fabrication capability.[87] This 4 cell-based micro-SLA can further lead the researchers and engineers to a better consequence in the field of bioprinting 3D cardiovascular tissue structures.

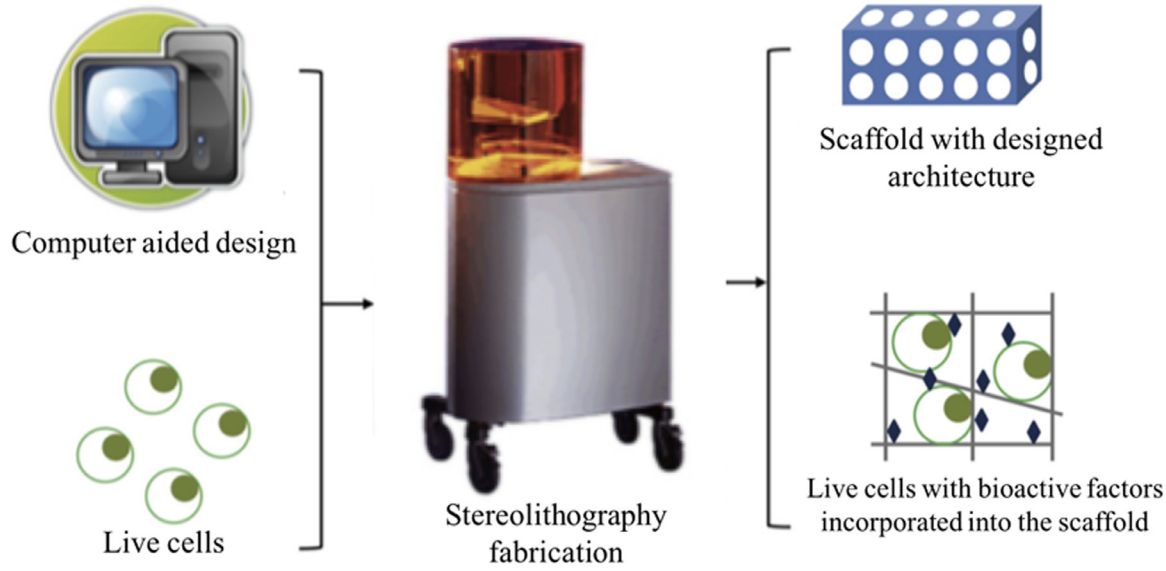

Fig 6. Overview of the design and fabrication of structures by stereolithography (SLA). Computer-aided design (CAD) is used to produce 3D models with desired architectures. Designed models are then sliced into a series of 2D images, which are transferred to the SLA device. By sequential photo-crosslinking, 3D scaffolds are built which are exact replicas of designed 3D models. Reproduction with permission from Ref.[84]

BIOINKS FOR CARDIAC TISSUE ENGINEERING

The utilization of hydrogels in tissue engineering is the current gold standard to mimic the 3D extracellular matrix found in native tissues, as they are biocompatible, biodegradable, and provide mechanical support to developing tissue. Crosslinked hydrogels are highly porous and allow cells to migrate and populate the scaffold and allow nutrients to be delivered to cells to meet their metabolic needs. In addition, because most hydrogels exhibit viscoelastic properties, they make an ideal candidate to serve as bioinks for the bioprinting process. The bioprinting of cardiac tissue most exploits the following biomaterials as bioinks:

Alginate. Alginate is a naturally occurring polysaccharide block copolymer derived from seaweed. Its linear molecular backbone is composed of (1,4)-linked β-D-mannuronate (M) and α-L-guluronate (G) residues in varying content and distribution along the length of the polymer.[88,89] Although the proportion of M to G residues varies, alginate can be more readily crosslinked as more G residues are present in the molecule along with divalent cations such as Ca^{2+}. Unfortunately, alginate is biologically inert and cannot facilitate cell adhesion on its own. With respect to cardiac tissue engineering, it has been elucidated that the immobilization of arginine-glycine-aspartate peptides on alginate scaffolds promote the generation of cardiac tissue as well as its preservation in culture in contrast to control alginate scaffolds that were not modified.[90] Thus, when considering this hydrogel as a bioink, one must consider coating the hydrogel with cell-adhesion moieties that support the development of cardiac tissue, such as heparin-binding peptide[91] or Matrigel.[92]

Although cells cannot directly attach to alginate scaffolds without the incorporation of cell-adhesion moieties, the biomaterial provides an excellent environment for human cardiac progenitor cells to be printed, differentiated, and developed into CMs.[93] In addition to providing a setting for CMs to grow, one advantage of using cell-laden alginate bioinks is the ability to easily tune the degree of crosslinking of the polymer, and as a consequence, the mechanical properties of the material. Some of the biomaterials that were utilized to 3D bioprint myocardial tissue include alginate,[41] as mentioned in the manuscript. Extrusion-based bioprinting with a cell-laden modified alginate bioink was implemented by Gaetani et al to produce constructs with human fetal CM progenitor cells.[74] Finally, Xu et al used the inkjet printing technique to develop a hierarchical design of functional cardiac pseudo tissues with a beating cell response, balanced by porosity for mass and structural support.[88] The tissue was engineered by printing layers of $CaCl_2$ into an alginate hydrogel precursor solution containing primary feline adult CMs, to induce crosslinking. The results of the study indicated that the cells adhered well to the alginate scaffold and also mimicked the native cardiac ECM.

Gelatin. Gelatin is a water-soluble protein resulting from the partial hydrolysis of collagen extracted from

the skin, bones, and connective tissues of animals like pigs and fish.[94-96] In the past, gelatin has been chemically modified by disulfide crosslinking,[97] photo-crosslinking,[98,99] and enzymatic crosslinking,[100,101] among others to enhance its mechanical properties. Owing to its biodegradability, biocompatibility, limited immunogenicity, and commercial availability at low costs, gelatin has found extensive applications in the field of cardiovascular tissue engineering.

Kumar et al[19] developed a novel visible light-crosslinkable gelatin-HA-based hydrogel to bioprint rectangular mono- and bilayered constructs, seeded with mouse MSCs in the former and C2C12 cardiac myoblasts and STO fibroblasts in the latter. The results of the study showed that the cells retained their viability and functionality in both cases after 5 days of culture showing that gelatin could be potentially used for the fabrication of cardiac tissues. The research was taken further when fibrin was incorporated into the CM-encapsulated gelatin-based hydrogel and used to 3D print patches that could mimic the native myocardium tissue, in terms of mechanical properties and cell-behavior.[20] The feasibility of using gelatin-based bioinks to form 3D constructs with cardiogenic potential was further illustrated by studies conducted by other researchers.[50,93,102] Results of MRI and histology studies further showed that there was a significant reduction in adverse remodeling and preservation of cardiac performance upon implantation of a gelatin-based cardiac construct into an infarcted mouse heart.[21] Further experiments by researchers at the University of Texas at El Paso also demonstrated that the gelatin-based hydrogel developed by Kumar et al[103], was superior to a Poly(ethylene glycol) diacrylate (PEGDA)-Eosin Y system, a widely accepted biomaterial developed by Bahney et al[104]], as the former exhibited a greater percentage of viable CMs when a live/dead assay was performed after 24 hours of culture, thereby validating the efficacy of f-gelatin as a promising material for the 3D printing of cardiac tissues. Briefly, the gelatin-based hydrogels when plated in 24 wells showed an average of 78% live cells, compared with the wells containing PEGDA-Eosin Y which could retain only 31% viable cells, when compared with cells cultured on 2D tissue culture polystyrene wells only (96% viable cells).[103]

Fibrin. Fibrin, a natural biodegradable polymer, has been investigated in cardiovascular tissue engineering and exhibited significant properties for 3D tissue structures.[105] Fibrin gel constructs complete autologous scaffold without the risk of immunogenic reactions as it can be derived from patients own blood plasma.[105,106] In addition, fibrin can be employed as gel, glue, or microbeads and it incorporates with various peptides, which results in increasing the local concentrations of growth factors and mimicking the natural tissue microenvironment.[106] As a tissue engineering scaffold, fibrin supports cell attachment, migration, proliferation, and the synthesis of the extracellular matrix through releasing platelet-derived growth factors and the transforming growth factor beta.[107]

Fibrin scaffold has provided important advantages for being used as cardiovascular structures like heart valves, cardiac muscles, aortic conduits, and blood vessels.[106,107] In an in vivo myocardial tissue regeneration approach, a silicone tubing was implanted around the femoral artery and vein of adult rats.[108] The silicone tube carried a suspension of fibrin gel and rat CMs. After 3 weeks, the tissue within the tube mimicked normal myocardial tissue.[108] In another approach, a valve conduit was fabricated using a suspension of myofibroblasts and fibrin gel (Fig 7).[105] A molding technique made of variable silicon layers was used to create the scaffold for the vascular wall and the cusp structures. After 4 weeks of culturing period, the electron microscopy images on the fibrin gel revealed a porous scaffold with well-developed extracellular matrix and organized collagen bundles without any nutrition problem.[105] The degradation of fibrin was controlled by adding aprotinin supplementation in the tissue culture medium. Aprotinin is a protease inhibitor which can stop or slow down the degradation of fibrin gel within a few days and ensure proper tissue development.[109] Additionally, molding of valve conduit and formation of different layer thickness were also possible using this gel.[105] In another study, different concentrations of aprotinin were used with fibrin in order to control the degradation of fibrin gel.[109] In this experiment, human aortic myofibroblasts were suspended in fibrin and cultured for 4 weeks. The 3D fibrin gel with aprotinin showed homogenous cell growth and confluent collagen production under electron microscopes, without any degradation or inflammatory reactions.[109] The results of this study demonstrated that the degradation of fibrin scaffold can be controlled by adjusting different aprotinin concentrations.

Fibrin, formed by the enzymatic polymerization of fibrinogen, plays an important role in the research of cardiac tissue engineering. Fibrin scaffold can support natural wound healing, controlled degradation, and better tissue development for cardiac tissue structures, while other biopolymers have significant disadvantages, such as PGA requires precoating for cell attachment and collagen is potentially immunogenic and also not biodegradable.[109] Fibrin gel also has some disadvantages like shrinkage, low mechanical stiffness, and gradual disintegration before the proper formation of the tissue structure.[106] These disadvantages can be avoided by cross-linking, optimizing the concentrations of fibrinogen, calcium ion (Ca^{2+}), and pH, or combining fibrin

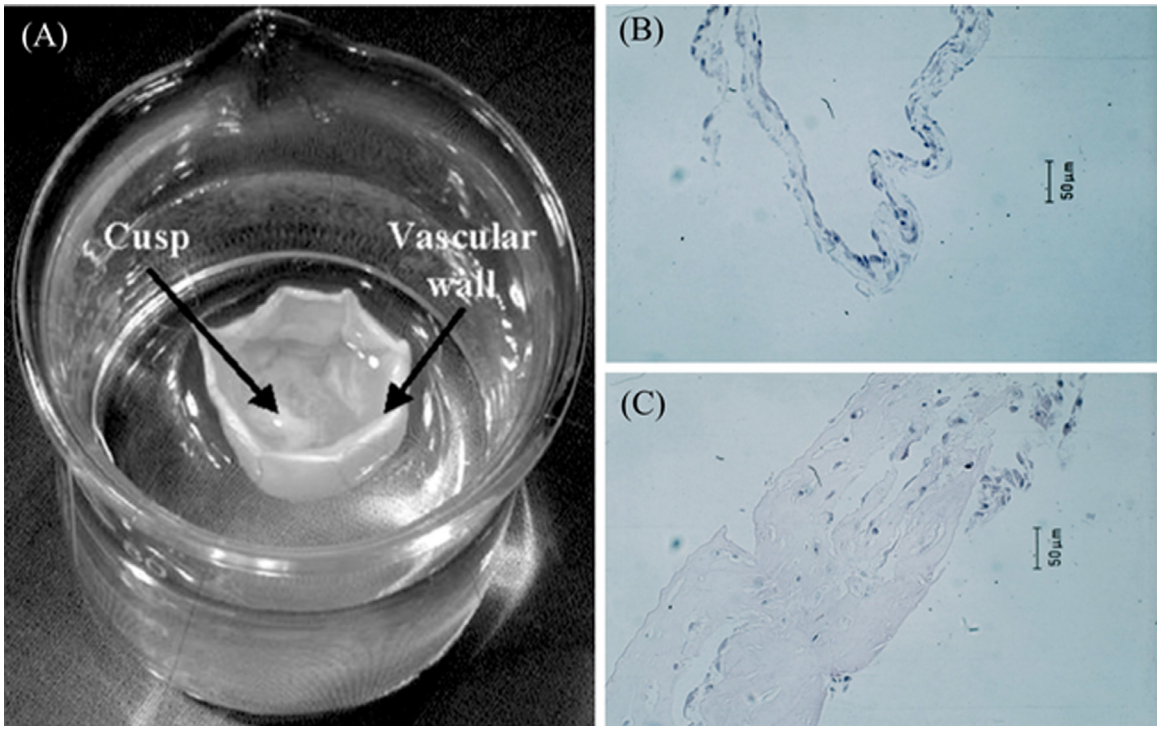

Fig 7. Fibrin and myofibroblasts cultured in different aprotinin concentration to control the degradation of fibrin gel. (A) model of a valve conduit based on a molded fibrin gel scaffold[105]; (B) fibrin gel cultured in lower concentration of aprotinin (5 mg), fibrin gel degraded and the structure appeared to be a thin cellular layer[109]; (C) fibrin gel cultured in high concentration of aprotinin (20 mg/mL), the cell-fibrin gel structure demonstrated a multilayer-structure with myofibroblasts surrounded by extracellular matrix.[109]

with other scaffolding polymers.[106] Although fibrin has some drawbacks, which need to be controlled, it has great promise in the fabrication of 3D cardiac tissue structures, due to its numerous advantages in supporting cell attachment, migration, and proliferation.

Collagen. Found naturally in the heart as type I one or type III, collagen gel may be used as a bioink for the printing of cardiac tissue. Because of its lack of stability after printing, the cross-linking of collagen is imperative to retain its structure and mechanical properties.[74] Although biomaterials can be used in the cross-linking of collagen, this can be achieved through a change in pH or in temperature.[110] This change in environment makes collagen stiffer through fibrillogenesis, yet takes a minimum of 30 minutes to occur, bringing about the use of other materials to speed up the process. Biomaterials capable of binding to collagen all while being biocompatible with cardiac cells include the plant derived cross-linkers tannin acid and proanthocyanidins. The cross-linking mechanism of both tannin acid and proanthocyanidins with collagen involves the formation of hydrogen bonds, which is made possible due to the abundance of proline in collagen.[111,112] Because of the high need for collagen throughout the scientific community, type I collagen can be obtained at an average of $10/mg with bovine connective tissue being the main source of this highly sought protein.[113]

The use of collagen for 3D bioprinting of cardiac tissue comes with numerous applications. For example, cardiac cells adhere and proliferate well within collagen, allowing for internal scaffold structures to be seeded.[114] This property has been used by Kreimendahl in the formation of blood vessels within a collagen scaffold and by Lee who used collagen as the bioink in the formation a network of capillaries connecting 2 vascular channels.[115] By providing a capillary network to 3D bioprinted cardiac tissue, oxygen and nutrients are able to reach cells enclosed by the scaffold. Another example is presented by Eschenhagen et al, who seeded CMs within collagen type I from rat tail in order to measure the isometric contraction of the cardiac tissue.[116] The successful shrinking of the collagen tissue due to mechanical forces demonstrates the capability of collagen to construct a contractible cardiac patch. In addition to its adherent and contractile properties, collagen contains a fibrous nature making it stiffer than most biomaterials. This characteristic was examined by Deborde and colleagues as they fabricated a collagen and elastin-based mitral valve scaffold by stabilizing type IV collagen through the use of penta−galloyl glucose.[116] Preventing

the degradation of collagen by collagenases, forms a robust and cytocompatible biomaterial necessary for maintaining the structure and function of a mitral valve. In addition to being 3D printable, collagen can be used to study different morphologic effects caused by a variety of diseases attributed to cardiac failure. For instance, of great concern is a stiffening of the heart following a myocardial infarction. This is thought to be caused by an increase in collagen I and III, leading to fibrosis and limiting contraction of the heart.[117] To study this phenomenon, our group developed collagen gels composed solely of collagen I and III solutions, which crosslink at neutral pH and 37°C. Ratios of collagen I:III were formulated, including 100% collagen I set as a control, a 9:1 ratio representative of a damaged heart, and a 3:1 ratio characteristic of healthy cardiac tissue. Under this set up it was observed that an increase in collagen I is highly attributed to cardiac fibrillogenesis postinfarction, hence a decrease in the amount of collagen I produced by cardiac fibroblasts could prevent future cardiac complications in patients (unpublished data).

Synthetic bioinks. Synthetic biomaterials are commonly used as alternatives to their natural counterparts in cardiac tissue engineering, with the ability of controlling the properties of the materials through well-known chemical reactions. An example includes polycaprolactone (PCL), whose melting temperature of 60°C allows it to be used for printing of cardiac scaffolds. A study by Ho et al saw the printing of carbon nanotubes (CNTs) with PCL for the engineering of cardiac tissue with results showing PCL was biodegradable and allowed proliferation of mouse myoblasts in vitro.[118] These findings highlight PCL as a biodegradable and biocompatible bioink that allows cardiac cells to grow and is able to degrade leaving only the newly grown cardiac tissue in place. Another synthetic material capable of being used as a bioink is polylactic acid (PLA). As shown by Kabirian et al, PLA can be bioprinted into tubular scaffolds for vascular grafts and shows high cell viability as well as slow degradation.[119] These are properties beneficial to the engineering of vascular grafts since the prolonged duration of PLA within the graft allows for structural support while cells align, and its high cell viability allows cells to withstand its long process. Synthetic bioinks are hence able to provide a durable framework while being biocompatible, allowing for the formation of much needed grafts for both cardiac implants and bypass surgeries.

POSTFABRICATION

Following 3D printing of an engineered cardiac tissue or scaffold, postfabrication methods are required to accustom the material to its new functions. Such methods are selected based on the various properties cardiac tissue requires for optimal performance, including those of contraction, reception and delivery of blood, and electrical signaling. Mechanical stimulation can be performed through continuous stretching and contraction of the material, as has been performed on a cardiac patch (Fig 8).[120] This ensures that the material has the capability of contracting and relaxing for long periods of time, all while maintaining its original morphology. This resistance to a permanent structural change is crucial, with the average resting heart of an adult being between 70−80 bpm.[121]

Limitations have complicated the development of cardiac tissue including oxygen diffusion, deep cell seeding within a scaffold, and cell attachment. To overcome these obstacles, perfusion techniques have been used to deliver nutrients throughout the bioprinted scaffold as well as for the seeding of cardiac cells (eg CMs, CFs, ECs, etc.).[122] As a result of perfusion cell seeding, cardiac cells become uniformly placed throughout the scaffold, allowing for improved cell survival and proliferation.[123,124]

Along with perfusion and mechanical stimulation, electrical conditioning is necessary for the initiation of muscle contraction. The stimulation of bioprinted cardiac tissue through electrical pulses has been shown to improve the conductive properties of cardiac cells and elicit a contraction rate characteristic of pacemaker cells.[125] Because cardiomyocyte contraction must occur simultaneously, electrical stimulation has been utilized to organize atrial and ventricular structure and attain an orchestrated beating of all CMs.[126,127] Desired characteristics of a functioning heart have been acquired through mechanical, perfusion, and electrical stimulation, suggesting postfabrication is needed for a fully functioning 3D bioprinted cardiac tissue to be constructed.

Numerous in vivo studies employing 3D printing to fabricate decellularized ECM scaffolds to treat myocardial infarction and restore cardiac functions have been conducted. Jang et al 3D printed prevascularized and functional multimaterial constructs from stem cell-laden decellularized extracellular matrix bioinks.[27] These patches were observed to promote strong vascularization and tissue matrix formation when implanted on infarcted rat hearts in vivo. The patterned patch also exhibited enhanced cardiac functions, reduced cardiac hypertrophy and fibrosis, increased migration from patch to the infarct area, neomuscle and capillary formation in addition to improving the cardiac functions.[27] Myocyte maturation has also been shown to improve significantly with more complex bioinks consisting of whole decellularized ECM.[22] Moreover, studies have also shown that decellularized ECM can fabricate biological scaffolds as a bioink to supply

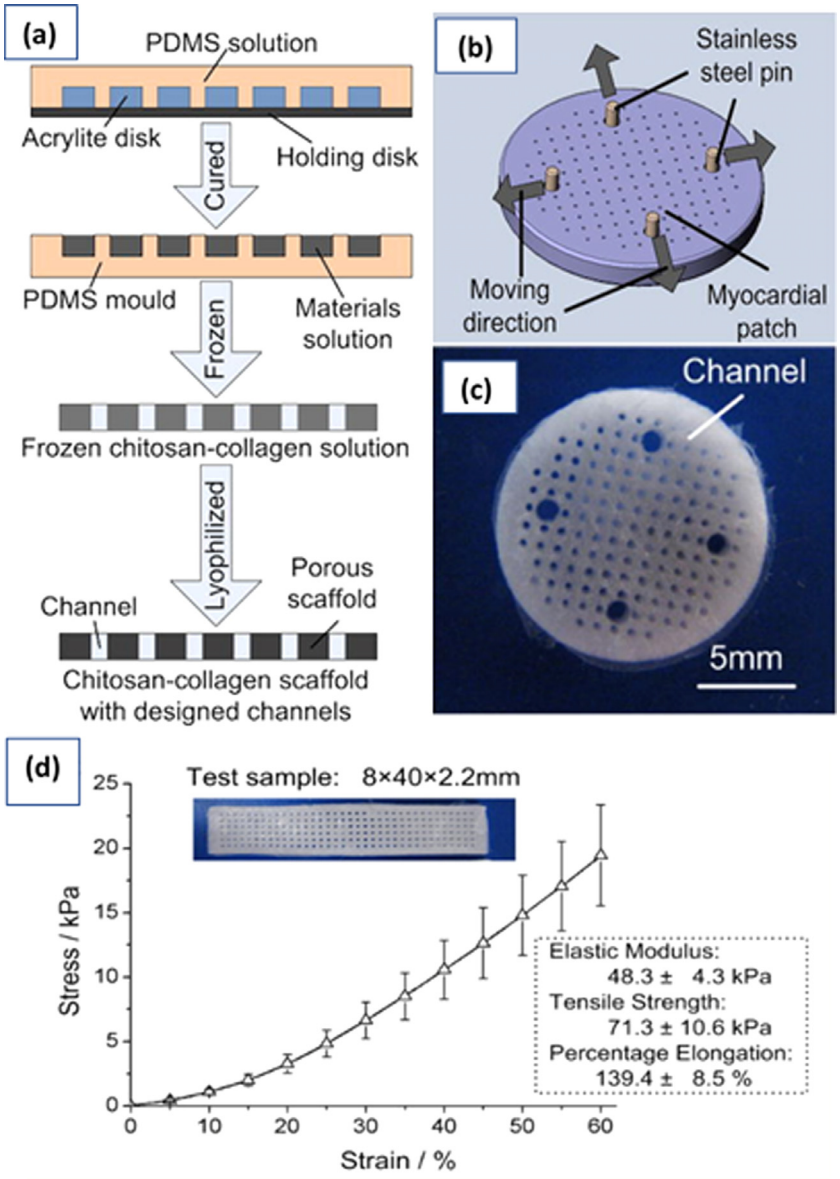

Fig 8. Formation and mechanical stimulation of chitosan-collagen scaffold. (a) Scheme for chitosan-collagen scaffold formation; (b) Scheme depicting mechanical stimulation applied to chitosan-collagen scaffold; (c) Chitosan-collagen scaffold; (d) Stress/strain graph. Reproduced with permission from Ref.[120]

biochemical cues more effectively compared to a polymer-based bioink for 3D printing.[22,128] However, further clinical studies utilizing decellularized ECM are required to be able to gain better insight into its potential to effectively treat myocardial infarction and translate them into human clinical trials. Table 1 summarizes each bioprinting technique, biomaterial, and conditioning technique.

Ong et al bioprinted tissue spheroids composed of human induced pluripotent stem cell-derived cardiomyocytes, fibroblasts, and endothelial cells into myocardial patches.[24,26] These patches were then implanted in vivo into a rodent model and demonstrated engraftment and vascularization, suggesting the therapeutic regenerative potential of this scaffold-free 3D bioprinting method.[24,26] Studies conducted by other groups have also demonstrated that a 3D bioprinted myocardium can promote vascularization and significant therapeutic effects, when implanted into animal hearts.[24,26,75,129,130]

SUMMARY AND FUTURE DIRECTIONS

Three-dimensional printing of cardiovascular constructs holds great promise in the development of

Table 1. The various bioprinting techniques, bioinks, and past fabrication techniques for cardiovascular tissue engineering of various structures are summarized along with relevant citations

Bioprinting technique	Applications	Features
Extrusion	Creation of thick myocardial constructs; heart valve formation; construction of blood vessels[73-75].	Viscoelastic bioinks; slow printing; medium cost; 3D; low resolution
Inkjet	Formation of blood vessels with HUVECS; 2D cardiac pseudo tissues[76-89].	Low viscosity suspension; fast printing; low cost; 2D; high resolution
Laser-assisted	Blood vessel formation; cardiac disease modeling; creation of cardiac tissues[90-97].	Hydrogel materials; medium speed; high cost; 3D; high resolution
Stereolithography	Creation of aortic valves; creation of implantable cardiac tissue with MSCs[100-111].	Light-sensitive polymers; fast printing; low cost; 3D; high resolution

Bioink	Applications
Alginate	Cardiac disease modeling; scaffold for CMs[112-117].
Gelatin	Scaffold for CMs and CFs[118-128].
Fibrin	Heart valves; cardiac muscle scaffold, blood vessel formation[129-133].
Collagen	Scaffold for CMs; formation of larger blood vessels and capillaries; construction of mitral valves; modeling heart disease such as fibrosis[134-141].
Synthetic	Scaffolds for CMs and tubular scaffolds for vascular construction[118,119]

Postfabrication	Applications
Mechanical	Physical stimulation by contracting and stretching out printed myocardial tissue.[120-121]
Electrical	Stimulation with electric fields to promote contraction of CMs.[125-127]
Perfusion	Delivery of nutrients and oxygen to thicker constructs; continuous shear stress on endothelium.[122-124]

clinically relevant tissues of size and integration of heterogenous cell types. Current techniques, technology, and materials limit engineering of only small units of functioning tissues. In order to develop complete organs, fundamental biological processes, better technology, and the advancement of material science need to be elucidated.

Although 3D bioprinting is a promising technology, whole organ bioprinting has remained elusive due to multiple challenges related to the inherent biology of the process, bioprinting technology, bioink material, and the postbioprinting maturation process.[131] In order to advance toward this goal, bioprinting of functional tissues tissue chips may be an intermediate stage toward achieving organ-level complexity in the future. In vitro fabrication of functional tissues is a complex phenomenon comprising an ordered arrangement of multiple cell types, including a multiscale network of vasculature, along with lymphatic vessels, neural, and muscle tissue for engineering cardiac tissues. Furthermore, these tissues need electrical pacing for their autonomous contraction, for which hydrogel-based scaffolds are not the best suited for electrical rhythm propagation through the newly regenerated tissues. Shin et al fabricated functional cardiac patches by culturing neonatal rat CMs onto carbon nanotube (CNT)-incorporated photo-cross-linkable GelMA hydrogels. The resultant cardiac patches showed excellent mechanical rigidity and electrophysiological functions. Specifically, myocardial tissues cultured on CNT-GelMA showed 3 times higher spontaneous synchronous beating rates and 85% lower excitation threshold, compared to those cultured on pristine GelMA hydrogels.[132] However, the in vivo biocompatibility of these synthetic CNT-based hydrogels is still far on the horizon. Also, extrapolating these outcomes to human iPSC-CMs is still a challenge.

Since the myocardium is a complex anatomic structure, recapitulating its complexity within engineered 3D functional constructs with tailored biological and mechanical properties, is one of the current scientific priorities in the field of regenerative medicine and tissue engineering. Bioprinting technology offers a great benefit in the categorized arrangement of cells or building tissue blocks in such a complex 3D microenvironment. In a recently published study by Maiullari F et al, multicellular constructs composed of HUVECs and iPSC-CMs were constructed. Briefly, cells were encapsulated within a hydrogel-based mixture containing alginate, Poly(ethylene glycol) (PEG)-Fibrinogen and extruded as cell-gel strands through a microfluidic printing head. Resulting 3D cardiac tissue constructs were composed of iPSC-derived CMs with a high orientation index imposed by the different defined

geometries and blood vessel-like shapes generated by HUVECs.[133] These 3D cardiac constructs may support the integration of the engineered cardiac tissue with host vasculature, when implanted in vivo.

The ratios of noncardiac cell types cultured along with CMs is also an issue that needs to be studied in depth, for optimization of a functional tissue engineered cardiac patch. Using a gelatin-fibrin based bioink,[20] we attempted to optimize the ratio for co-culturing 3 different types of cardiac cells, namely CMs, CFs, and, ECs, enabling them to couple in the 3D printed construct. To demonstrate proof of feasibility we showed heterocellular coupling of CMs and fibroblasts,[20] alongside others' work that showed the association between endothelial cells with cardiomyocytes[134,135] in human heart tissue samples. Our approach will mirror the known connections of CMs to networks of fibroblasts, and ECs; that co-exist in the cardiac tissue wall, in vivo. To our knowledge, this is the only study where heterocellular coupling has been accomplished between different cell types via bioprinting.

Design of the scaffold used for bioprinting a cardiac patch is also extremely important as this can affect mechanical rigidity and fidelity of the scaffold after in vivo implantation. In our recently published study we compared 2 different structures, namely a lattice and a rectangular-sheet that were bioprinted using the same bioink composition.[136] Results showed that the lattice structure posed as an enhanced scaffold with structural rigidity and optimal porosity, allowing cells to proliferate more, compared to the rectangular-sheet structure.

Another aspect to account for is cell interaction at the interface when using 2 dissimilar cells types to bioprint multilayered tissue. The materials used for constructing interface or junction should specifically promote cell interaction and allow cells from different layers to synergize within one composite tissue construct. In our recently published study, we used 3D bioprinting to demonstrate heterocellular crosstalk between C2C12 myocytes and STO fibroblasts at the junction or interface between the top and bottom printed cell sheets layered one atop another using an f-gelatin-based bioink.[19]

Although several researchers have considered bioprinting of tissue constructs, the fabrication of scale-up tissues with a high volumetric oxygen-consumption rate for cardiac tissue is still a challenge. One major roadblock is to bioprint vascularized thick tissues, from simple to complex. Since it is difficult to print capillaries at the submicron scale using current technology, an alternative could be to create the pattern for fabricating the macrovasculature and then leave nature to create the capillaries. To this end, 2 alternative approaches have been considered: (1) indirect bioprinting by utilizing a fugitive ink that is removed by thermally induced de-crosslinking, leaving a vascular network behind;[137] and (2) direct bioprinting of a vasculature network in a tubular shape.[138]

In addition to additive manufacturing of complex tissue constructs, subtractive manufacturing techniques for making vascularized tissue constructs are much needed. Recently, in collaboration with Dr Katja Michaels's group, a 34 amino acid long collagen-like peptide with photoreactive nitroindoline groups built into the peptide backbone was generated, which enables the breakdown of the peptide into smaller peptide fragments upon illumination with near UV light by a one photon process, or with femtosecond laser light of 710 nm by a two photon process.[139,140] Unlike photopolymerization, this photolysis does not propagate through the material, but occurs only at the site of illumination, opening doors for a localized decomposition within a macroscopic material. We expect that a block of a hydrogel composed of these photoreactive collagen-like peptides can be shaped with light to carve out tunnels to provide a biomimetic scaffold that can support a vasculature and tissue growth. This approach may ultimately be useful for engineering cardiac tissues with a vasculature network.

Apart from the existing types of bioprinting technologies available, some have unique capabilities such as the The BioAssemblyBot which is capable of not only 3D bioprinting but also contour printing. The 6-axis robotic arm of the BioAssemblyBot, capable of nearly 360° movement and reach, offers considerable flexibility in performance to the user. Coupled with interchangeable end-effectors, or tools, the arm performs an array of different fabrication tasks, including 3D bioprinting and transfer between build stations. Up to 8 of the many different available tools can be employed in a single fabrication run, thereby promoting complex tissue assembly and task execution. In addition, the robotic arm enables both additive and contour printing, an aspect of manufacturing necessary for printing complex biological structures on complex and curved surfaces.

Bioreactor systems are a vital component used to provide a tissue-specific physiological in vitro environment during tissue maturation, postprinting. Based on the bioreactor technology, various connective tissue systems can be incubated in vitro, in dynamic environments such as spinner flasks and perfusion systems.[141] By using computation fluid dynamics, the flow in a perfusion bioreactor system can be further optimized. Additional hydrostatic pressure, shear stress, and turbulence were monitored during variations in flow rate to correlate the relations and approximate the final process properties.[142] In this manner, computational analysis can be applied to promote enhanced understanding

of the mechanical stimuli and environment, which are vital for the establishment of the tissue functions.

In essence, the advent of three-dimensional bioprinting technology has allowed the field of cardiac tissue engineering to progress toward clinically relevant treatments for CVD. Although the field and technology are still in their infancy, 3D bioprinting holds great promise in recapitulating structures such as the myocardium, blood vessels, and heart valves that mimic those found in the native heart. The development of new bioinks and printers capable of high-resolution construction would further progress the field, and end-goal of engineering complete organs. In addition, research in stem cell biology and high throughput generation of cardiac cells would allow organs to be constructed on demand. Further research must also be conducted on the implantation and integration of engineered constructs. Nonetheless, 3D bioprinting is a powerful tool that holds great promise into translating engineered cardiovascular tissues from the lab to bedside.

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REFERENCES

1. World Health Organization. Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region. Geneva; 2018.
2. Duan Bin. State-of-the-Art Review of 3D Bioprinting for Cardiovascular Tissue Engineering. Ann Biomed Eng 2017;45 (1):195–209.
3. Gaudino Mario, Benedetto Umberto, Fremes Stephen, et al. Radial-Artery or Saphenous-Vein Grafts in Coronary-Artery Bypass Surgery. N Engl J Med 2018;378(22):2069–77.
4. Alraies M Chadi, Eckman Peter. Adult heart transplant: indications and outcomes. J Thoracic Dis 2014;6(8):1120–8.
5. Rizwan A Manji, Menkins Alan H, Ekser Burcin, et al. Porcine bioprosthetic heart valves: The next generation. Am Heart J 2012;164(2):177–85.
6. Pashneh-Tala Samand, MacNeil Sheila, Claeyssens Frederik. The Tissue-Engineered Vascular Graft—Past, Present, and Future. Tissue Eng B 2016;22(1):68–100.
7. Page Aravinda, Messer Simon, Large Stephen R. Heart transplantation from donation after circulatory determined death. Ann. Cardiothoracic Surg. 2018;7(1):75–81.
8. Verheugt Freek WA. Anticoagulation in patients with mechanical heart valves: follow the guidelines! Neth Heart J 2015;23:109–10.
9. Pajaro Octavio E, Jaroszewski Dawn E, Scott Robert L, et al. Antibody-Mediated Rejection in Heart Transplantation: Case Presentation with a Review of Current International Guidelines. J Transplant 2011;2011:1–7.
10. Bouten CVC, Dankers PYW, Driessen-Mol A, Pedron S, Brizard AMA, Baaijens FPT. Substrates for cardiovascular tissue engineering. Adv Drug Deliv. Rev 2011;63:221–41.
11. Jawad H, Ali NN, Lyon AR, et al. Myocardial tissue engineering: a review. J Tissue Eng Regen Med 2007;1:327–42.
12. Zhu Junmin, Marchant Roger E. Design properties of hydrogel tissue-engineering scaffolds. Expert Rev Med Devices 2011;8 (5):607–26.
13. Siepe Matthias, Akhyari Payam, Lichtenberg Artur, Schlensak Christian, Beyersdorf Freidhelm. Stem cells used for cardiovascular tissue engineering. Eur J Cardio-thorac Surg 2008;38:242–7.
14. Bursac Nenad. Cardiac Tissue Engineering Using Stem Cells. IEEE Eng Med Biol Mag 2009;28(2):80–9.
15. Duelen Robin, Sampaolesi Maurillo. Stem Cell Technology in Cardiac Regeneration: A Pluripotent Stem Cell Promise. BioMedicine 2017;16:30–40.
16. Gaetani Roberto, Doevendans Peter A, Metz Corina HG, et al. Cardiac tissue engineering using tissue printing technology and human cardiac progenitor cells. Biomaterials 2012;33(6):1782–90.
17. Jakab Karoly, Narotte Cyrille, Damon Brook, et al. Tissue engineering by self-assembly of cells printed into topologically defined structures. Tissue Eng A 2008;14(3):413–21.
18. Jakab Karoly, Marga Francoise, Norotte Cyrille, et al. Tissue engineering by self-assembly and bio-printing of living cells. Biofabrication 2010;2(2):1–34.
19. Kumar Shweta Anil, Allen Shane C, Tasnim Nishat, et al. The applicability of furfuryl-gelatin as a novel bioink for tissue engineering applications. J Biomed Mater Res B: Appl Biomater 2018;107(2):314–23.
20. Kumar Shweta Anil, Alonzo Matthew, Tasnim Nishat, et al. A visible light crosslinkable, fibrin-gelatin based bioprinted cardiac patch with human cardiomyocytes and fibroblasts. ACS Biomater Sci Eng; 2018[submitted for publication].
21. Gaetani Roberto, Feyen Dries AM, Verhage Vera, et al. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. Biomaterials 2015;61:339–48.
22. Pati Falguni, Jang Jinah, Ha Dong-Heon, et al. Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun 2014;5(3935):1–11.
23. Atmanli Ayhan, Domain Ibrahim J. Generation of aligned functional myocardial tissue through microcontact printing. J Visual Exp 2013;73:1–7.
24. Ong Chin Siang, Fukunishi Takuma, Zhang Huaitao, et al. Biomaterial- Free Three-Dimensional Bioprinting of Cardiac Tissue using Human Induced Pluripotent Stem Cell Derived Cardiomyocytes. Scientific Rep 2017;7(1):1–11.
25. Bejleri Donald, Streeter Benjamin W, Nachlas Aline LY, et al. A Bioprinted Cardiac Patch Composed of Cardiac-Specific Extracellular Matrix and Progenitor Cells for Heart Repair. Adv Healthcare Mater 2018;7(23):1–13.
26. Ong Chin Siang, Fukunishi Takuma, Nashed Andrew, et al. Creation of cardiac tissue exhibiting mechanical integration of

spheroids using 3D bioprinting. J Visual Exp 2017;2017(125): e55438.
27. Jang Jinah, Park Hun-Jun, Kim Seok-Won, et al. 3D printed complex tissue construct using stem cell-laden decellularized extracellular matrix bioinks for cardiac repair. Biomaterials 2017;112:264–74.
28. Vukicevic Marija, Mosadegh Bobak, Min James K. Little Stephen H., Cardiac 3D Printing and Its Future Directions. JACC Cardiovasc Imaging 2017;10(2):171–84.
29. Bücking Thore M, Hill Emma R, Robertson James L. From medical imaging data to 3D printed anatomical models. PLoS One 2017;12(5):1–10.
30. Salgo Ivan S. Three-dimensional echocardiography. J Cardiothoracic Vasc. Anesth 1997;11(4):506–16.
31. Vukicevic Marija, Mosadegh Bobak, Min James K, Little Stephen H. Cardiac 3D printing and its future directions. JACC: Cardiovasc Imaging 2017;10(2):171–84.
32. Rozner Mark A, Burton Allen W, Kumar Ashok. Pacemaker complication during magnetic resonance imaging. J Am College Cardiol 2005;45(1):161–2.
33. Lusic Hrvoje, Grinstaff Mark W. X-ray-computed tomography contrast agents. Chem Rev 2012;113(3):1641–66.
34. Rajiah Prabhakar, Tandon Animesh, Greil Gerald F, Abbara Suhny. Update on the role of cardiac magnetic resonance imaging in congenital heart disease. Curr Treatment Otions Cardiovasc Med 2017;19(2):2.
35. Kato Kayoto, Ishiguchi Tsuneo, Maruyama Kunihiro, Naganawa Shinji, Ishigaki Takeo. Accuracy of plastic replica of aortic aneurysm using 3D-CT data for transluminal stentgrafting: experimental and clinical evaluation. J Comput Assist Tomogr 2001;25(2):300–4.
36. Knox K, Kerber Charles W, Singel SA, Bailey MJ, Imbesi SG. Rapid prototyping to create vascular replicas from CT scan data: making tools to teach, rehearse, and choose treatment strategies. Catheter Cardiovasc Interv 2005;65(1):47–53.
37. Kim Michael S, Hansgen Adam R, Wink Onno, Quaife Robert A, Carroll John D. Rapid prototyping: a new tool in understanding and treating structural heart disease. Circulation 2008;117 (18):2388–94.
38. Schievano Silvia, Migliavacca Francesco, Coats Louise, et al. Percutaneous pulmonary valve implantation based on rapid prototyping of right ventricular outflow tract and pulmonary trunk from MR data. Radiology 2007;242(2):490–7.
39. Sodian Ralf, Weber Stefan, Markert Mathias, et al. Stereolithographic models for surgical planning in congenital heart surgery. Ann Thorac Surg 2007;83(5):1854–7.
40. Markl Michael, Schumaker Ralf, Kuffer Jurg, Bley Thorsten A, Hennig Jurgen. Rapid vessel prototyping: vascular modeling using 3T magnetic resonance angiography and rapid prototyping technology. MAGMA 2005;18(6):288–92.
41. Farooqi Kanwal M, Sengupta Partho P. Echocardiography and three-dimensional printing: sound ideas to touch a heart. J Am Soc Echocardiogr 2015;28(40):398–403.
42. Binder Thomas M, Moertl Deddo, Mundigler Gerald, et al. Stereolithographic biomodeling to create tangible hard copies of cardiac structures from echocardiographic data: in vitro and in vivo validation. J Am Coll Cardiol 2000;35(1):230–7.
43. Olivieri Laura J, Krieger Axel, Loke Yue-Hin, et al. Three-dimensional printing of intracardiac defects from three-dimensional echocardiographic images: feasibility and relative accuracy. J Am Soc Echocardiogr 2015;28(4):392–7.
44. Noecker Angela M, Chen Ji-Feng, Zhou Qun, et al. Development of patient-specific three-dimensional pediatric cardiac models. Asaio J 2006;52(3):349–53.
45. Ripley Beth, Kelil Tatiana, Cheezum Michael K, et al. 3D printing on classic CT assists anatomic visualization prior to transcatheter aortic valve replacement. J Cardiovasc Comp Tomogr 2016;10(1):28–36.
46. Bartel Thomas, Rivard Andrew, Jimenez Alejandro, Mestres Carlos A, Müller Silvana. Medical three-dimensional printing opens up new opportunities in cardiology and cardiac surgery. Eur. Heart J 2017;39(15):1246–54.
47. Valverde Israel, Gomez Gorka, Gonzalez Antonio, et al. Three-dimensional patient-specific cardiac model for surgical planning in Nikaidoh procedure. Cardiol. Young 2015;25(4):698–704.
48. Mottl-Link Sibylle, Hübler Michael, Kühne Titus, et al. Physical models aiding in complex congenital heart surgery. Ann. Thoracic Surg 2008;86(1):273–7.
49. Dababneh Amer B, Ozbolat Ibrahim T. Bioprinting Technology: A Current State-of-the-Art Review. J Manuf Sci Eng 2014;136:61016-1-61016-11.
50. Zhang Yu Shrike, Arneri Andrea, Bersini Simone. Bioprinting 3D microfibrous scaffolds for engineering endothelialized myocardium and heart-on-a-chip. Biomaterials 2016;110:45–59.
51. Cui Xiaofeng, Gao Guifang, Yonezawa Tomo, Dai Guohao. Human Cartilage Tissue Fabrication Using Three-Dimensional Inkjet Printing Technology. J Vis Exp 2014;10(88):1–5.
52. Xu Tao, Zhao Weixin, Zhu Jian-Ming, et al. Complex heterogeneous tissue constructs containing multiple cell types prepared by inkjet printing technology. Biomaterials 2013;34:130–9.
53. Calvert Paul. Printing cells. Science 2007;318(5848):208–9.
54. Le Hue P. Progress and Trends in Inkjet Printing Technology. J Imaging Sci Technol 1998;42(1):49–62.
55. Stachowiak Jeanne C, Richmond David L, Li Thomas H, Brochard Francoise, Fletcher Daniel A. Inkjet Formation of Unilamellar Lipid Vesicles for Cell-Like Encapsulation. Lab Chip 2009;9(14):2003–9.
56. Singh Madhusudan, Haverinen Hanna M, Dhagat Parul, Jabbour Ghassan E. Inkjet printing-process and its applications. Adv Mater 2010;22(6):673–85.
57. Boland Thomas, Xu Tao, Damon Brook, Cui Xiaofeng. Application of Inkjet Printing to Tissue Engineering. Biotechnol J 2006;1(9):910–7.
58. Xu Tao, Jin Joyce, Gregory Cassie, Hickman James, Boland Thomas. Inkjet Printing of Viable Mammalian Cells. Biomaterials 2005;26(1):93–9.
59. Phillippi Julie A, Miller Eric, Weiss Lee, Huard Johnny, Waggoner Alan, Campbell Phil. Microenvironments Engineered by Inkjet Bioprinting Spatially Direct Adult Stem Cells Toward Muscle- and Bone-Like Subpopulations. Stem Cell Res 2008;26(1):127–34.
60. Lee Wonhye, Debastis Jason C, Lee Vivian K, et al. Multi-layered culture of human skin fibroblasts and keratinocytes through three-dimensional freeform fabrication. Biomaterials 2009;30(8):1587–95.
61. Cui Xiaofeng, Boland Thomas. Human microvasculature fabrication using thermal inkjet printing technology. Biomaterials 2009;30(31):6221–7.
62. Lee Vivian K, Dai Guohao. Three-dimensional bioprinting and tissue fabrication: prospects for drug discovery and regenerative medicine. Adv Health Care Technol 2015;2015 (1):23–35.
63. Xu Tao, Baicu Catalin, Aho Michael, Zile Michael, Boland Thomas. Fabrication and characterization of bio-engineered cardiac pseudo tissues. Biofabrication 2009;1(3):1–12.
64. Clemson University. Inkjet Technology For Organ Printing. ScienceDaily; 2007.

65. Gruene Martin, Delwick Andrea, Koch Lothar, et al. Laser Printing of Stem Cells for Biofabrication of Scaffold-Free Autologous Grafts. Tissue Eng C: Methods 2011;17(1):79–87.
66. Duocastella Marti, Colina Monica, Fernández-Pradas JM, et al. Study of the laser-induced forward transfer of liquids for laser bioprinting. Appl Surf Sci 2007;253(19):7855–9.
67. Riester Dominik, Budde J, Gach Christian, Gillner A, Wehner Martin. High speed photography of laser induced forward transfer (LIFT) of single and double-layered transfer layers for single cell transfer. J Laser Micro Nanoeng 2016;11(2):199–203.
68. Gruene Martin, Unger Claudia, Koch Lothar, Deiwick Andrea, Chichkov Boria. Dispensing pico to nanolitre of a natural hydrogel by laser-assisted bioprinting. Biomed Eng Online 2011;10(9):1–11.
69. Guillotin Bertrand, Souquet Agnes, Catros Sylvain, et al. Laser assisted bioprinting of engineered tissue with high cell density and microscale organization. Biomaterials 2010;31 (28):7250–6.
70. Koch Lothar, Kuhn Stefanie, Sorg Heiko, et al. Laser Printing of Skin Cells and Human Stem Cells. Tissue Eng C: Methods 2010;16(5):ahead of print.
71. Nahmias Yaakov, Schwartz Robert E, Verfaillie Catherine M, Odde David J. Laser-guided direct writing for three-dimensional tissue engineering. Biotechnol Bioeng 2005;95 (2):129–36.
72. Ozbolat Ibrahim T, Moncal Kazim K, Gudapati Hemanth. Evaluation of bioprinter technologies. Additive Manuf 2017;13: 179–200.
73. Gruene Martin, Plaufm Michael, Hess Christian, et al. Laser Printing of Three-Dimensional Multicellular Arrays for Studies of Cell–Cell and Cell–Environment Interactions. Tissue Eng C: Methods 2011;17(10):973–82.
74. Zisch Andreas H, Zeisberger Steffen M, Ehrbar Martin, et al. Engineered fibrin matrices for functional display of cell membrane-bound growth factor-like activities: Study of angiogenic signaling by ephrin-B2. Biomaterials 2004;25(16):3245–57.
75. Gaebel Ralf, Ma Nan, Liu Jun, et al. Patterning human stem cells and endothelial cells with laser printing for cardiac regeneration. Biomaterials 2011;32(35):9218–30.
76. Rider Patrick, Kacarevic Zeijka Peric, Alkidani Said, Retnasingh Sujith, Barbeck Mike. Bioprinting of tissue engineering scaffolds. J Tissue Eng 2018;9:1–16.
77. Bishop Elliot S, Mostafa Sami, Pakvasa Mikhail, et al. 3-D bioprinting technologies in tissue engineering and regenerative medicine: Current and future trends. Genes Dis 2017;4 (4):185–95.
78. Jang Jinah, 3D bioprinting and in vitro cardiovascular tissue modeling. Bioengineering. 4(3): p. 71.
79. Gilon Dan, Cape Edward G, Handschumacher Mark D, et al. Effect of three-dimensional valve shape on the hemodynamics of aortic stenosis: three-dimensional echocardiographic stereolithography and patient studies. J Am College Cardiol 2002;40 (8):1479–86.
80. Sodian Ralf, Loebe Matthias, Hein Andreas, et al. Application of stereolithography for scaffold fabrication for tissue engineered heart valves. ASAIO J 2002;48(1):12–6.
81. Melhem Molly R, Park Jooyeon, Knapp Luke, et al. 3D printed stem-cell-laden, microchanneled hydrogel patch for the enhanced release of cell-secreting factors and treatment of myocardial infarctions. ACS Biomater Sci Eng 2016;3(9):1980–7.
82. Wang Zongjie, Abdulla Raafa, Parker Benjamin, et al. A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks. Biofabrication 2015;7(4):1–10.
83. Borovjagin Anton V, Ogle Brenda, Berry Joel, Zhang Jianyi. From microscale devices to 3D printing: advances in fabrication of 3D cardiovascular tissues. Circ. Res 2017;120 (1):150–65.
84. Lin Hang, Zhang Dongning, Alexander Peter G, et al. Application of visible light-based projection stereolithography for live cell-scaffold fabrication with designed architecture. Biomaterials 2013;34(2):331–9.
85. Tzeng Jy-Jiunn, Hsiao Yi-Ting, Wu Yun-Ching, et al. Synthesis, Characterization, and Visible Light Curing Capacity of Polycaprolactone Acrylate. BioMed Res. Int 2018;2018:1–8.
86. Grogan Shawn P, Chung Peter H, Soman Pranav, et al. Digital micromirror device projection printing system for meniscus tissue engineering. Acta Biomater 2013;9(7):7218–26.
87. Miri Amir K, Nieto Daniel, Iglesias Luis, et al. Microfluidics-Enabled Multimaterial Maskless Stereolithographic Bioprinting. Adv Mater 2018;30(27):1–9.
88. Lee Kuen Yong, Mooney David J. Alginate: Properties and Biomedical Applications. Prog Polymer Sci 2012;37 (1):106–26.
89. Eiselt Petra, Yeh Julia, Latvala Rachel K, Shea Lonnie D, Mooney David J. Porous Carriers for Biomedical Applications Based on Alginate Hydrogels. Biomaterials 2000;21 (19):1921–7.
90. Shachar Michal, Tsur-Gang Orna, Dvir Tal, Leor Jonathan, Tal Dvir, Cohen Smadar. The effect of immobilized RGD peptide in alginate scaffolds on cardiac tissue engineering. Acta Biomater 2011;7(1):152–62.
91. Sapir Yulia, Kryukov Olga, Cohen Smadar. Integration of multiple cell-matrix interactions into alginate scaffolds for promoting cardiac tissue regeneration. Biomaterials 2011;32 (7):1838–47.
92. Stowers Ryan S, Allen Shane C, Suggs Laura. Dynamic phototuning of 3D hydrogel stiffness. PNAS 2015;112(7):1953–8.
93. Gaetani Roberto, Doevendans Peter A, Metz Corina HG, et al. Cardiac tissue engineering using tissue printing technology and human cardiac progenitor cells. Biomaterials 2012;33(6):1782–90.
94. Drury Jeanie L, Mooney David J. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 2003;24(24):4337–51.
95. Kang Hye-Won, Tabata Yasuhiko, Ikada Yoshito. Fabrication of porous gelatin scaffolds for tissue engineering. Biomaterials 1999;20(14):1339–44.
96. Lee Kuen Y, Mooney David J. Hydrogels for tissue engineering. Chem Rev 2001;101(7):1869–80.
97. Shu Xiao Zheng, Liu Yanchun, Palumbo Fabio, Prestwich Glenn D. Disulfide-crosslinked hyaluronan-gelatin hydrogel films: a covalent mimic of the extracellular matrix for in vitro cell growth. Biomaterials 2003;24(21):2834–3825.
98. Zhao Xin, Lang Qi, Yildirimer Lara, et al. Photocrosslinkable gelatin hydrogel for epidermal tissue engineering. Adv Healthcare Mater 2016;5(1):108–18.
99. Mazaki Tetsuro, Shiozaki Yasuyuki, Yamane Kentaro, et al. A novel, visible light-induced, rapidly cross-linkable gelatin scaffold for osteochondral tissue engineering. Scientific Rep 2014;4 (4457):1–10.
100. Yang Gang, Xiao Zhenghua, Xen Xiaomei, et al. Enzymatically crosslinked gelatin hydrogel promotes the proliferation of adipose tissue-derived stromal cells. Peer J 2016;4:1–22.
101. Lemes Gislaine F, Marchiore Nicolli Grecco, Moreia Thaysa F, et al. Enzymatically crosslinked gelatin coating added of bioactive nanoparticles and antifungal agent: Effect on the quality of Benitaka grapes. LWT-Food Sci Technol 2017;84:175–82.

102. Ajay Tijore, Irvine Scott Alexander, Sarig Udi, et al. Contact guidance for cardiac tissue engineering using 3D bioprinted gelatin patterned hydrogel. Biofabrication 2018;10(2):1–14.
103. Anil Kumar Shweta. Exploration and optimization of biomaterials and cells required for the fabrication of a 'cardiac patch. University of Texas at El Paso; 2019[Unpublished doctoral dissertation].
104. Bahney CS, Lujan TJ, Hsu CW, et al. Visible light photoinitiation of mesenchymal stem cell-laden bioresponsive hydrogels. Eur. Cells Mater 2011;22(43):43–55.
105. Jockenhoevel Stefan, Zund Gregor, Hoerstrup Simon P, et al. Fibrin gel–advantages of a new scaffold in cardiovascular tissue engineering. Eur. J Cardio-thoracic Surg 2001;19(4):424–30.
106. Ahmed Tamer AE, Dare Emma V, and Hincke Max, Fibrin: a versatile scaffold for tissue engineering applications. Tissue Eng B: Reviews. 14(2):199-215.
107. Sierra D, Saltz R. Surgical Adhesives and Sealants: Current Technology and Applications, 48. Lancaster, PA: Technomic Publishing Company, Inc; 1996. p. 1461–6.
108. Birla Ravi K, Borschel Gregory H, Dennis Robert G, Brown David L. Myocardial engineering in vivo: formation and characterization of contractile, vascularized three dimensional cardiac tissue. Tissue Eng 2005;11(5-6):803–13.
109. Ye Qing, Zund Gregor, Benedikt Peter, et al. Fibrin gel as a three dimensional matrix in cardiovascular tissue engineering. Eur. J Cardio-Thoracic Surg 2000;17(5):587–91.
110. Ahn Hee-Jin, Khalmuratova Roza, Park Su A, et al. Serial analysis of tracheal restenosis after 3D-printed scaffold implantation: recruited inflammatory cells and associated tissue changes. Tissue Eng Regen Med 2017;14(5):631–9.
111. Hagerman Ann E, Butler Larry G. The Specificity of Proanthocyanidin-Protein Interactions. J Biol Chem 1981;256 (9):4494–7.
112. Frazier Richard A, Deaville Eddie R, Green Rebecca J, et al. Interactions of tea tannins and condensed tannins with proteins. J Pharm 2010;51(2):490–5.
113. Brodsky Barbara, Kaplan David L. Shining Light on Collagen: Expressing Collagen in Plants. Tissue Eng A 2013;19(13-14):1499–501.
114. Kreimendahl Franziska, Köpf Marius, Thiebes Anja Lena. 3D-Printing and Angiogenesis: Tailored Agarose-Type I Collagen Blends Comprise 3D Printability and Angiogenesis Potential for Tissue Engineered Substitutes. Tissue Eng C: Methods 2017;23(10):604–15.
115. Lee Vivian K, Lanzi Allison M, Haygan Ngo, et al. Generation of multi-scale vascular network system within 3D hydrogel using 3D bio-printing technology. Cell Mol Bioeng 2014;7 (3):460–72.
116. Eschenhagen Thomas, Fink Christine, Remmers Ute, et al. Three-dimensional reconstitution of embryonic cardiomyocytes in a collagen matrix: a new heart muscle model system. FASEB J 1997;11:683–94.
117. Pauschinger Matthias, Doerner Andrea, Remppis Andrew, et al.] Differential myocardial abundance of collagen type I and type III mRNA in dilated cardiomyopathy: Effects of myocardial inflammation. Cardiovasc Res 1998;37(1):123–9.
118. Ho Chee Meng Benjamin, Mishra Abhinay, Lin Pearlyn Teo Pei, et al. 3D Printed Polycaprolactone Carbon Nanotube Composite Scaffolds for Cardiac Tissue Engineering. Macromol Biosci 2016;17(4):1–9.
119. Fatemeh Kabirian, Ditkowski Bartek, Zamanian Ali, Heying Ruth, Mozafari Masoud. An innovative approach towards 3D-printed scaffolds for the next generation of tissue-engineered vascular grafts. Materials Today: Proceedings 2018;5 (7):15586–94.
120. Zhang Ting, Wan Leo Q, Xiong Zhuo, et al. Channelled scaffolds for engineering myocardium with mechanical stimulation. J Tissue Eng Regen Med 2012;6(9):748–56.
121. Hart John. Normal resting pulse rate ranges. J Nurs Educ Pract 2015;5(8):95–8.
122. Maidhof Robert, Marsano Anna, Lee Eun Jung, Vunjak-Novakovic Gordana. Perfusion seeding of channeled elastomeric scaffolds with myocytes and endothelial cells for cardiac tissue engineering. Biotechnol Prog 2010;26(2):565–72.
123. Radisic Milica, Yang Liming, Boublik Jan, et al. Medium perfusion enables engineering of compact and contractile cardiac tissue. AJP Hear Circ Physiol 2004;286(2):H507–16.
124. Tandon Nina, Taubman Alanna, Cimetta Elisa, Saccenti Laetitia, Vunjak-Novakovic Gordana. Portable bioreactor for perfusion and electrical stimulation of engineered cardiac tissue. In: In: Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society, EMBS, 2013:6219–23.
125. Mohamed Mohamed A, Islas Jose, Schwartz Robert, Birla Ravi. Electrical stimulation of artificial heart muscle: A look into the electrophysiologic and genetic implications. ASAIO 2017;63 (6):333–41.
126. Chi Neil C, Bussen Markus, Brand-Arzamendi Koroboshka, et al. Cardiac conduction is required to preserve cardiac chamber morphology. Proc Natl Acad Sci USA 2010;107 (133):14662–7.
127. Eng George, Lee Benjamin W, Protas Lev, et al. Autonomous beating rate adaptation in human stem cell-derived cardiomyocytes. Nat Commun 2016;7(10312):1–10.
128. Pati Falguni, Ha Dong-Heon, Jang Jinah, et al. Biomimetic 3D tissue printing for soft tissue regeneration. Biomaterials 2015;62:164–75.
129. Gaetani Roberto, Feyen Dries AM, Verhage Vera, et al. Epicardial application of cardiac progenitor cells in a 3D-printed gelatin/hyaluronic acid patch preserves cardiac function after myocardial infarction. Biomaterials 2015;61:339–48.
130. Gao Ling, Kupfer Molly E, Jung Jangwook P, et al. Myocardial tissue engineering with cells derived from human-induced pluripotent stem cells and a native-like, high-resolution, 3-dimensionally printed scaffold. Circulation Research 2017;120 (8):1318–25.
131. Ozbolat Ibrihim T, Yu Yin. Bioprinting toward organ fabrication: challenges and future trends. IEEE Trans Biomed Eng 2013;60(3):691–9.
132. Shin Su Ryon, Jung Sun Mi, Zalabany Momen, et al. Carbon-nanotube-embedded hydrogel sheets for engineering cardiac constructs and bioactuators. ACS Nano 2013;7(3):2369–80.
133. Maiullari Fabio, Costantini Marco, Milan Marika, et al. A multi-cellular 3D bioprinting approach for vascularized heart tissue engineering based on HUVECs and iPSC-derived cardiomyocytes. Sci Rep 2018;8(1):1–15.
134. Condorelli G, Borello U, De Angelis L, et al. Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: implications for myocardium regeneration. Proc Natl Acad Sci USA 2001;98(19):10733–8.
135. Gherghiceanu Mihaela, Popescu Laurentiu M. Cardiac telocytes - their junctions and functional implications. Cell Tissue Res 2012;348(2):265–79.
136. Anil Kumar Shweta, Tasnim Nishat, Dominguez Erick, et al. A Comparative Study of a 3D Bioprinted Gelatin-Based Lattice and Rectangular-Sheet Structures. Gels 2018;4(3):1–12.

137. Kolesky David B, Truby Ryan L, Gladman A Sydney, et al. 3D bioprinting of vascularized, heterogeneous cell-laden tissue constructs. Adv Mater 2014;26(19):3124–30.
138. Hinton Thomas J, Jallerat Quentin, Palchesko Rachelle N, et al. Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. Sci Adv 2015;1(9):1–10.
139. Hatch Kevin A, Ornelas Alfredo, Williams Kaitlyn N, et al. Photolysis of a peptide with N-peptidyl-7-nitroindoline units using two-photon absorption. Biomed Opt Express 2016;7(11):4654–9.
140. Ornelas Alfredo, Williams Kaitlyn N, Hatch Kevin A, et al. Synthesis and characterization of a photocleavable collagen-like peptide. Org. Biomol. Chem 2018;16(6):1000–13.
141. Rosser Jordan, Thomas Daniel J. Bioreactor processes for maturation of 3D bioprinted tissue. Reconstruct Surg 2018: 191–215.
142. Gharravi Anneh Mohammad, Orazizadeh Mahmoud, Hashemitabar Mahmoud. Fluid-induced low shear stress improves cartilage like tissue fabrication by encapsulating chondrocytes. Cell Tissue Bank 2016;17(1):117–22.