TISSUE ENGINEERING: Part B
Volume 00, Number 00, 2021

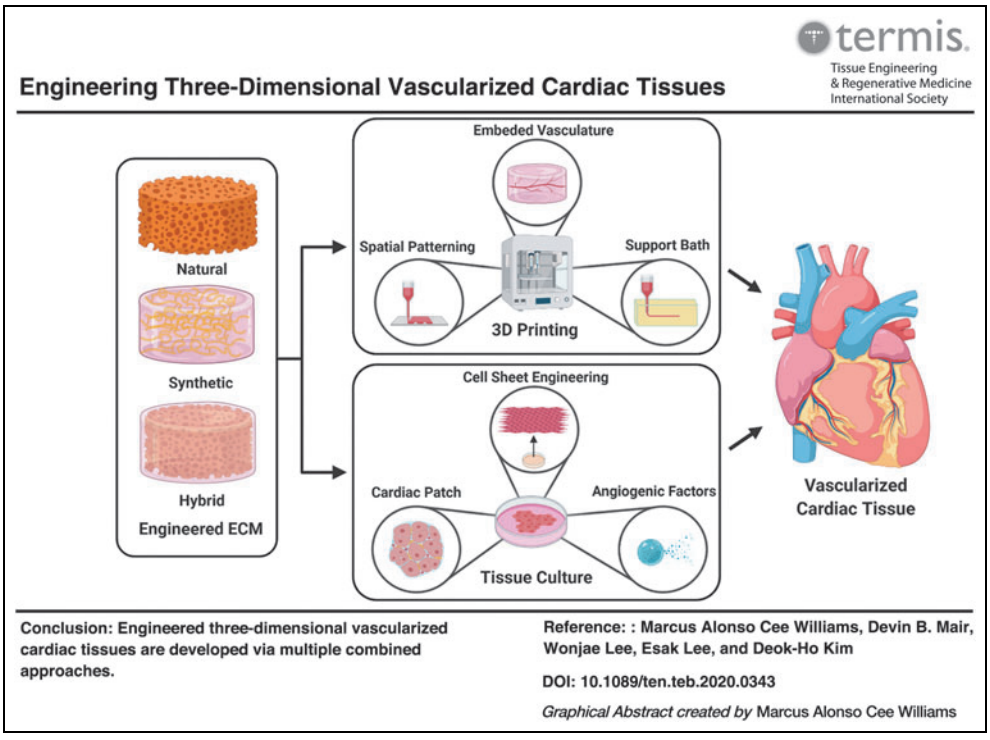
DOI: 10.1089/ten.teb.2020.0343

termis
Tissue Engineering
& Regenerative Medicine
International Society

Engineering Three-Dimensional Vascularized Cardiac Tissues

Marcus Alonso Cee Williams, BS,[1] Devin B. Mair, BS,[1] Wonjae Lee, PhD,[2]
Esak Lee, PhD,[3] and Deok-Ho Kim, PhD[1,4]

Heart disease is one of the largest burdens to human health worldwide and has very limited therapeutic options. Engineered three-dimensional (3D) vascularized cardiac tissues have shown promise in rescuing cardiac function in diseased hearts and may serve as a whole organ replacement in the future. One of the major obstacles in reconstructing these thick myocardial tissues to a clinically applicable scale is the integration of functional vascular networks capable of providing oxygen and nutrients throughout whole engineered constructs. Without perfusion of oxygen and nutrient flow throughout the entire engineered tissue not only is tissue viability compromised, but also overall tissue functionality is lost. There are many supporting technologies and approaches that have been developed to create vascular networks such as 3D bioprinting, co-culturing hydrogels, and incorporation of soluble angiogenic factors. In this state-of-the-art review, we discuss some of the most current engineered vascular cardiac tissues reported in the literature and future directions in the field.



Keywords: engineered cardiac tissue, 3D printed vasculature, vascularized cardiac tissues, angiogenesis, regenerative medicine, cardiac patch

[1]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland, USA.
[2]Department of Neurosurgery, Stanford School of Medicine, Stanford, California, USA.
[3]Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, New York, USA.
[4]Department of Medicine, Johns Hopkins School of Medicine, Baltimore, Maryland, USA.

Impact Statement

The field of cardiac tissue engineering is rapidly evolving and is now closer than ever to having engineered tissue models capable of predicting preclinical responses to therapeutics, modeling diseases, and being used as a means of rescuing cardiac function following injuries to the native myocardium. However, a major obstacle of engineering thick cardiac tissue remains to be the integration of functional vasculature. In this review, we highlight seminal and recently published works that have influenced and pushed the field of cardiac tissue engineering toward achieving vascularized functional tissues.

Introduction

Heart failure is estimated to affect at least 26 million individuals globally.[1] Ischemic heart disease is the most common cause of heart failure and one of the main causes of death worldwide, claiming the lives of over 9 million people worldwide in 2016 alone.[2–4] Even though there are treatments currently administered for ischemic heart disease[2,5] and overall heart failure,[6–10] there is no disease-modifying therapy other than organ transplantation. However, in addition to problems such as surgical complications and organ rejection, heart transplantation is inherently limited by the number of total available functional hearts.[11,12] The field of cardiac tissue engineering has opened up the possibility of overcoming these problems by reconstructing and replacing original tissues or portions of them to restore their original function. The ultimate goal of cardiac tissue engineering is to resolve the desperate need for organ donors by engineering functional hearts at a clinically relevant scale for implantation. Immune-mediated rejection of new organs and foreign objects is one of many challenges that must be overcome by further developing biocompatible and patient-specific tissues.[13–15] Recent advances in stem cell biology and biomaterials are now expected to solve many of these associated challenges by providing patient-derived pluripotent stem cells and through further refinement of advanced biomaterials.[16–19]

More recently, as methodologies of tissue engineering have advanced to recapitulate cardiac microtissue structures more elaborately, the restoration of vascular networks throughout engineered tissues has emerged as a major interest and challenge within the field.[20–22] This is because the incorporated cells need to be within 100–200 μm of functional vasculature to secure oxygen and nutrient supply and diffuse out waste products, which are prerequisites to maintain cellular viability and organ-specific functionality.[23,24] In particular, cardiac-specific vasculature forms synchronously with cardiac tissue and extensively interacts with it through a variety of paracrine, autocrine, and endocrine factors for organ development and mature functionality.[25–30] It is becoming increasingly clear that vasculature in cardiac tissue is of grand importance at both a transcriptional level and in cardiac function as a whole.[31–35] In this review, we discuss techniques being used to integrate functional cardiac tissues into *in vivo* models. In addition, we address recent advances in engineered vascularized cardiac tissues utilizing three-dimensional (3D) printing strategies. Finally, we discuss current limitations, challenges, and the future directions and perspectives in which the field of engineering cardiac tissues progresses toward.

Engineered Biomaterials to Recapitulate the Native Extracellular Matrix

One critical challenge in the field of tissue engineering is to engineer biomaterials that provide physiologically relevant microenvironments to incorporated cells.[36] Cells sensitively respond to their immediate surroundings, the microenvironment, such as spatial arrangement, mechanochemical cues, nutrient availability, and gas exchange.[37–46] To achieve organ-level functionality, the biomaterial must recapitulate physiological properties of the native extracellular matrix (ECM) so that the incorporated cells exhibit their original phenotypes and the resultant tissues function as observed *in vivo*. In addition, the scaffold biomaterials also need to exhibit mechanical stability to support cells in a relevant 3D spatial arrangement. Compared to two-dimensional (2D) tissue culture on plastic dishes, many engineered organ systems have successfully provided tissue-specific 3D microenvironments for the incorporated cells and obtained targeted functionalities.[22,47–52]

Hydrogels have been most widely used as an ECM scaffold for engineered cardiac tissues. Hydrogels are classified into three categories: natural, synthetic, and hybrid. Naturally derived hydrogels generally comprised ECM derived-components (such as decellularized ECM [dECM]), polysaccharides (such as alginate), and peptide chains (such as collagen or spider silk).[50,53–58] These are optimal for recapitulating ECM-mediated biochemical signaling as seen *in vivo* and are susceptible to biodegradability, which may be desirable when engineering cardiac tissue implants to aid in integration into the *in vivo* tissue.[59,60] Alternatively, synthetic hydrogels are often biologically inert, but allow for fine-tuning to recapitulate key aspects of the targeted native microenvironment.

The core components of the synthetic hydrogels used in many recent reports commonly comprised polyethylene glycol (PEG) or its modified derivatives.[61] Tunable chemical/photo-crosslinkable moieties can be integrated into hydrogel polymeric networks for the fine-tuning of substrate physiochemical properties.[57,62] Biochemical cues such as growth factors (i.e., vascular endothelial growth factor: VEGF[63]), binding domains (i.e., fibronectin[64]), proteins (i.e., laminin[63]), or peptide chains (i.e., arginine-aspartic acid [RGD][65]) can be covalently bound or blended into a synthetic hydrogel to guide cell fate and cellular organization, and increase biocompatibility and tune biodegradability.[66] While synthetic hydrogels offer greater abilities to customize specific cell-substrate interactions, it is questionable how closely synthetic hydrogels mimic native ECM. Therefore, hybrid hydrogels, a blend of both synthetic and natural hydrogels, are of interest, in that, they can encompass the advantages of both natural and synthetic

hydrogels. Hybrid hydrogels have the structural flexibility of synthetic hydrogels, while providing native ECM components.[67–74] These may be the most advanced biomimetic hydrogels to date, but are still far from truly recapitulating the highly complex *in vivo* native ECM.

Conventional Tissue Culture-Based Methodologies for the Development of Vascularized Cardiac Tissues

Many conventional tissue culture-based methods are being utilized to develop vascularized cardiac tissues. Vascularized cardiac tissues are useful not only for understanding disease pathology but also for meeting the clinical need for transplantation. In this section, we discuss several methods of developing vascularized cardiac tissue, focusing on methods that utilize 2D and 3D conventional culture systems that integrate both physical and biochemical cell-cell and cell-ECM interactions.

Engineering transplantable tissues through coculture methods

One interesting approach is the coculturing of tissue-specific progenitor cells with endothelial cells (ECs) to facilitate cell-cell interactions and mediate integration of host vasculatures in transplanted tissues.[70] The interactions between host cells and transplanted biomaterials have significant effects on integration of host vasculatures and angiogenesis.[75–79] This has led the field to focus on engineering tissues that have biomaterial and cell types, with physiological architecture, which allow further integration into host tissue upon transplantation (Fig. 1A).[80–85] Cardiac patches have been generated by culturing cells in low adhesion plates with varying cardiomyocyte (CM):human umbilical vein endothelial cells (HUVEC):mouse embryonic fibroblast ratios of 1:0:0, 1:1:0, and 1:1:0.5.[86] These cardiac patches were transplanted into rodent hearts and observed after 1 week. Of all three ratios, it was only found that one was viable (1:1:0.5). Interestingly, this particular ratio was reported to have formed viable $CD31^+$ endothelial networks resembling *in vivo* morphological vascular networks (Fig. 2A). In addition, the 1:1:0.5 tissues were observed to integrate into the host myocardial vasculature with lumens containing leukocytes and red blood cells, which were validated through analysis of Ter-199, an erythrocyte lineage marker, expression (Fig. 2B).

Cardiac patches with multiple cell types have also been fabricated using fibrin gel scaffolds containing both induced pluripotent stem cell-derived CMs (iPSC-CM) and vascular pericytes in a myocardial infarct (MI) model.[87] MI was achieved by permanent ligation of the left anterior descending coronary artery LAD, and cardiac patches containing either iPSC-CM only or iPSC-CM and vascular pericytes were transplanted into the infarct area. It was observed that the iPSC-CM and vascular pericyte patches were able to recruit host vasculature and remain viable over a 4-week period (Fig. 2C, D). In comparison to the iPSC-CM-only cardiac patch, the iPSC-CM and vascular pericyte patch treatment group had an increase in ejection fraction at the 1-week mark and fractional shortening at both the 1- and 4-week time points, indicating rescue of cardiac function. The concept of a tricultured transplantable cardiac patch was explored in a study using human CM, EC, and embryonic fibroblasts in biodegradable porous scaffolds composed of PLLA/PLGA.[82] Following 2 weeks of culture, it was reported that the engineered cardiac tissue was able to spontaneously contract, at which point, it was transplanted into the anterior wall of the left ventricle of rats. Two weeks following transplantation, there was significantly more host vascularization in the tricultured cardiac tissues compared to a control CM-only tissue quantified by average blood vessels present. Injection of florescent microspheres into coronary circulation revealed perfusion into both rat heart and the transplanted scaffold, demonstrating integration of host vasculature into transplanted tissues. Most interestingly, fibroblasts within the transplanted cardiac tissue were identified within the vascular wall and stained positively for αSMA, indicating differentiation into vascular mural cells, such as smooth muscle cells, exhibiting a more mature vessel morphology.

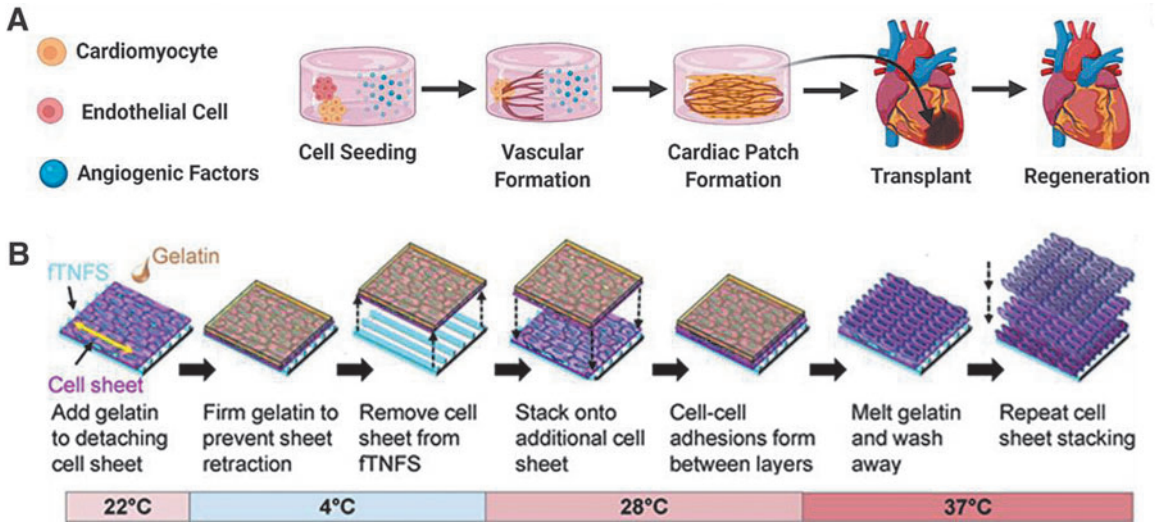


FIG. 1. Cardiac tissues can be cultured in numerous ways, which allow for their subsequent functionalization and transplantation. **(A)** Hydrogels with embedded cardiac-specific cell types and angiogenic growth factors can generate functional vascular cardiac patches that can be subsequently transplanted into diseased hearts. **(B)** An example of cardiac cell sheeting using thermosensitive gelatin for stacking several layers of cell sheets for mechanical stability and subsequent transplantation.[93]

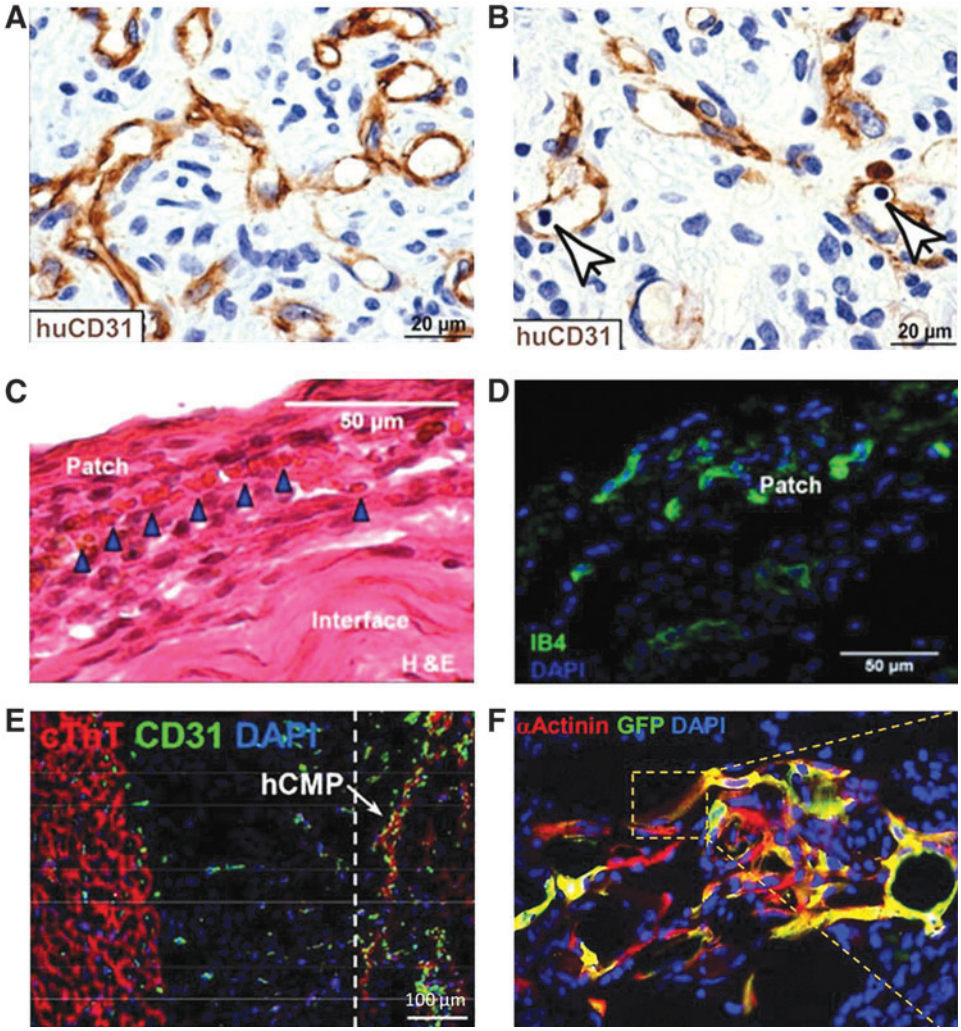


FIG. 2. Cardiac patches demonstrate the ability to integrate with host vasculatures **(A)** 1-week post implantation in a rat MI model vasculature within the cardiac patch stains positive for human $CD31^+$ endothelial networks.[86] **(B)** Vessel-like structures within the cardiac patch contain leukocytes (*white arrows*) demonstrating functional integration into host vasculature.[86] **(C)** H&E staining of the cardiac patch postimplantation reveals the presence of red blood cells within the patch (*blue arrows*).[87] **(D)** Integration of host vasculature identified by the presence of cells staining positive for isolectin B4 (IB4) within the cardiac patch.[87] **(E)** Implanted cardiac patch (hCMP) located to the *right* of the *white dashed line* was identified by visualization of engineered iPSC-CM expressing CD31 and cardiac troponin I (cTnT).[88] **(F)** Maturing iPSC-CM with distinct sarcomeric structures were visualized by staining for coexpression of GFP and α-actinin.[88] iPSC-CM, induced pluripotent stem cell-derived cardiomyocyte; MI, myocardial infarct.

In a recent study, a tricultured cardiac patch was developed using human iPSC-CM, iPSC-EC, and iPSC-smooth muscle cells (iPSC-SMC) within a fibrin-cased scaffold.[88] Following 1 day of implantation into the scaffold, it was observed that iPSC-CM were able to synchronously depolarize within the scaffold. Functionality of the cardiac patch *in vitro* was assessed 7 days following seeding in the fibrin scaffold, and it was observed that intracellular coupling of the iPSC-CM was similar to that of the left ventricle of rabbits and could reliably be placed in a broad range of intervals (450–1000 ms). To validate the efficacy of the cardiac patch in regenerative medicine applications, the authors moved toward engrafting the cardiac patch into a porcine model of MI. In this model, the LAD was occluded for 60 minutes, reperfused, and the cardiac patch was subsequently engrafted into the region of infarct. Following 4 weeks of observation, the recipient group had statistically significantly improved cardiac function compared to controls, along with reduced wall stress and infarct size. In addition, endogenous factors secreted by the patch improved cell proliferation and neovascularization of the embedded iPSC-EC (Fig. 2E, F). Proteomic analysis of the cardiac tissue following 4 weeks with the tricultured cardiac patch revealed an interesting stabilization of sarcomeric protein phosphorylation, suggesting that the cardiac patch had either prevented or reversed phosphorylation of key sarcomeric regulatory proteins that occur following MI. This unexpected, but interesting finding was hypothesized to have had a role in the improved cardiac function in the cardiac patch recipient group. As evidence of the works discussed in this section, cardiac patches containing multiple cell types found in native myocardium show improved functionality and rescue cardiac function in MI models. In addition, the inclusion of multiple cell types has improved recruitment of host vasculatures, which may aid in rescuing cardiac function along with ensuring long-term viability of the implanted grafts. iPSC-derived tissues seem to have the greatest effect on both vascularization of the region of infarct and overall cardiac function, which may be attributed to cell signaling pathways (e.g., angiogenesis, vasculogenesis, and cell cycle regulation)[89,90] in relatively immature tissues.

Cardiac cellular sheets

Cell sheeting capitalizes on cell-cell interactions in monolayer culture systems to obtain vascularized cardiac tissues through stacking monolayers (Fig. 1B).[91–95] In this system, CM monolayers are cultured on a temperature-responsive polymer, poly(N-isopropylacrylamide). Cells adhere to this polymer at physiological temperature, but

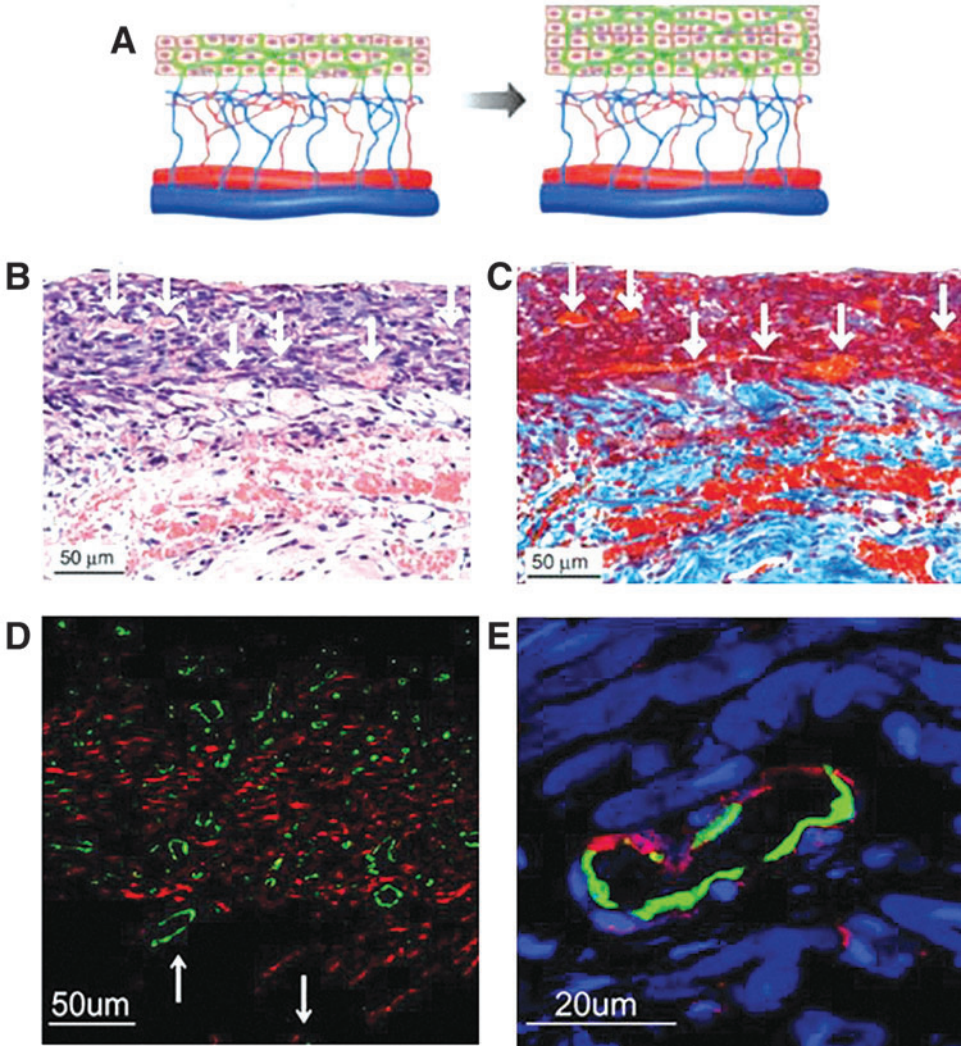


FIG. 3. Cell sheet engineering has been explored as a potential option for generating transplantable vascular cardiac tissues. **(A)** Cardiac cell sheets have been generated, layered, and cultured within vascular bioreactors to influence prevascularization before transplantation.[92] **(B, C)** H&E and Azan staining show evidence of cardiac sheets containing perfusable vasculature capable (*white arrows*) of supporting the engineered tissue.[92] **(D)** Transplanted cardiac cell sheet containing GFP expressing ECs stained with isolectin B4 demonstrates fused vasculature from cardiac cell sheet to host MI model.[99] **(E)** Cardiac patch containing GFP expressing ECs stained for troponin T (*red*) shows transplanted cardiac cell sheet vascularizing host myocardium in rat MI model.[99]

detach as a sheet at lower temperatures without the need for enzymatic processes that disrupt cell-cell and cell-surface adhesion.[96] This method reliably creates cardiac cell sheets that contain elongated sarcomeres and established gap junctions, and recruit vasculatures when implanted *in vivo*, while maintaining long-term viability and cardiac-specific functionality. Engineered layered cardiac cell sheets showed promise in morphological integration into host myocardium and improved cardiac function and recovery in *in vivo* MI models (Fig. 3A–C).[92,97,98] While these studies proved the potential for cardiac sheets in regenerative medicine, they still had yet to mimic the microenvironment of the native myocardium or integrate neovasculature. In an effort to address this shortcoming, cardiac cell sheets were co-cultured in varying relative ratios with EC (Fig. 3D, E).[99] When analyzed *in vitro,* cardiac sheets co-cultured with EC had significantly elevated secretion of VEGF, basic fibroblast growth factor, and hepatocyte growth factor in comparison to cell sheets composed of CM alone. When the cocultured cardiac sheets were transplanted into an MI rat model *in vivo*, there was a significant increase in fractional shortening and a decrease of cardiac fibrosis at the region of infarct. In addition, embedded EC were able to connect with host capillaries at the periphery of the cardiac cell sheet. In a more recent study, iPSC-CM, vascular EC, and vascular mural cells were cocultured within cell sheets.[100] In this study, cells were cultured on gelatin-coated thermosensitive culture dishes as opposed to poly(N-isopropylacrylamide) where cell sheets could release at physiological temperature. These sheets were transplanted into porcine MI models and it was observed that there was integration of host vasculature and formation of capillary beds within the grafted cell sheet. The cell sheet recipient group exhibited significantly larger ejection fractions and fractional shortening in comparison to the nontreated control group, indicating rescue of cardiac function. In addition, there was significantly less fibrosis in the group treated with cell sheets compared to the control. These data indicate that the cardiac cellular sheets are useful for improvement of cardiac function, vascularization, and potential long-term viability.

Angiogenic Factors and Their Incorporation into Cardiac Tissue Models

During development, tissues become vascularized *de novo* through angiogenesis, where EC remodel the ECM and form new capillary sprouts, which gives rise to a new functional circulatory system from existing blood vessels.[101,102] As

Table 1. Various Growth Factors Involved in Angiogenesis and Their Method of Action

Growth factor	*Activity*
VEGF	A signaling protein involved in vasculogenesis and angiogenesis through binding of VEGF receptors.[102,103]
PDGF	A growth factor that regulates blood vessel formation, blood vessel growth, vascular smooth muscle cell proliferation, and chemotaxis through receptor tyrosine kinase signals.[103,149]
SDF-1	A strongly chemotactic growth factor that directs formation of large blood vessels in fetal development, as well as in adult angiogenesis through recruitment of endothelial progenitor cells from bone marrow.[102]
IGF-1	A prosurvival growth factor that stabilizes nascent vessel formation, enhancing angiogenesis.[150]
HGF	A factor that regulates cell growth, motility, and morphogenesis. It acts as a mitogen for vascular EC, but not vascular smooth muscle cells, furthering angiogenesis.[102]
bFGF	The bFGF stimulates EC to secrete proteases to degrade the vessel basement membrane, allowing cells to invade the surrounding matrix, proliferate, and form neovessels.[102,151]
MCP-1	An angiogenic chemokine that activates VEGF downstream to further angiogenesis.[152]
PMA	The PMA increases production of collagenase and allows EC to invade through the basement membrane and form further vessels.[153]
Substance P	The Substance P is mitogenic for endothelial and vascular smooth muscle cells, promoting angiogenesis.[105,154]
S1P	A blood-borne lipid mediator that acts on G protein-coupled receptors, such as S1P receptors. It stimulates EC proliferation and migration, and formation of lumen-containing vessels.[155]

bFGF, basic fibroblast growth factor; EC, endothelial cell; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor 1; MCP-1, monocyte chemoattractant protein-1; PDGF, platelet-derived growth factor; PMA, phorbol 12-myristate 13-acetate; S1P, sphingosine-1-phophate; SDF-1, stromal cell-derived factor-1; VEGF, vascular endothelial growth factor.

discussed in Section 3, this process can be influenced by cell-cell interactions, but is also subject to signaling through angiogenic growth factors (Table 1).[85,102–105] EC will respond to gradients of one or a combination of growth factors and directionally sprout into the ECM, forming new capillaries toward the source of growth factors.[49]

In a seminal work, an approach was taken to engineer vascular cardiac tissue utilizing an alginate scaffold seeded with a mixture comprising Matrigel, mouse left ventricular CM, and growth factors stromal cell-derived factor-1, insulin-like growth factor-1 (IGF-1), and VEGF.[106] The patch was cultured for 48 h and then implanted into rat omentum for 7 days. Seven days following implantation, the cardiac patch was removed, and it was observed that there was extensive vascularization of the patch in comparison to control patches lacking growth factors. The prevascularized cardiac patches were then evaluated in an *in vivo* rat MI model and the degree of dyskinesis (irregular movement of the myocardium outward during systole[107]) was reduced in comparison to nontreated controls. This validated the functionality of the cardiac patch because increasing degrees of dyskinesis is a predictor of mortality following MI in patients.[108]

In a more direct approach, endogenous cell signaling was controlled by modulating VEGF expression in transduced myoblasts engrafted directly into an area of MI in an *in vivo* murine model.[109] Skeletal myoblasts transfected to express VEGF were cultured with rat CM within porous poly(glycerol sebacate) scaffolds. The patches were then engrafted into areas of MI and examined 4 weeks after implantation. It was reported that the VEGF expressing cardiac patches showed significantly more vasculatures in the area of infarct in comparison to non-VEGF expressing controls and were also able to induce angiogenesis into the underlying myocardium. This concept has been expanded on in more recent studies that have engineered biocompatible materials capable of controlled release of angiogenic factors over extended periods of time to recruit vascular ECs and stem cells.[110,111] While angiogenic factors have shown great promise in being therapeutic for MI and ischemic heart disease, their effects on single cell and systemic pathology are less understood. In an effort to engineer a system capable of safely releasing growth factors over extended periods of time to induce neovascularization in a localized region, VEGF and platelet-derived growth factor were encapsulated in poly(D,L-lactide-co-glycolide) acid and porous silica nanoparticles that were subsequently integrated into an electrospun gelatin scaffold.[111] When the scaffold with embedded growth factors was implanted subcutaneously in a murine model for 21 days, it was observed that there was an increase in αSMA and $CD31^+$ cells in the implant area, indicating enhanced neovascularization and vessel maturation. In addition, in the growth factor-embedded scaffold, qPCR revealed an increase in proangiogenic factors, further providing evidence that the scaffold was capable of influencing vascularization in a localized region. In a similar study, electrospun scaffolds containing embedded IGF-1 and substance P were fabricated as cardiac patches to influence neovascularization in an MI murine model.[110] In the electrospun cardiac patches, it was observed that patches containing both IGF-1 and substance P implanted in infarcted myocardium contained more $CD31^+$ vessels and isolectin B4-positive capillaries in comparison to other controls containing only one growth factor or none at all. These findings may suggest that this combination of growth factors may also act a synergistic pair when attempting to revascularize and heal damaged myocardium.

3D Bioprinting for Reconstructing Cardiac Tissue Constructs

In recent years, 3D printing technology has become increasingly advanced and reliable as an option for manufacturing complex tissues. 3D printing allows one to

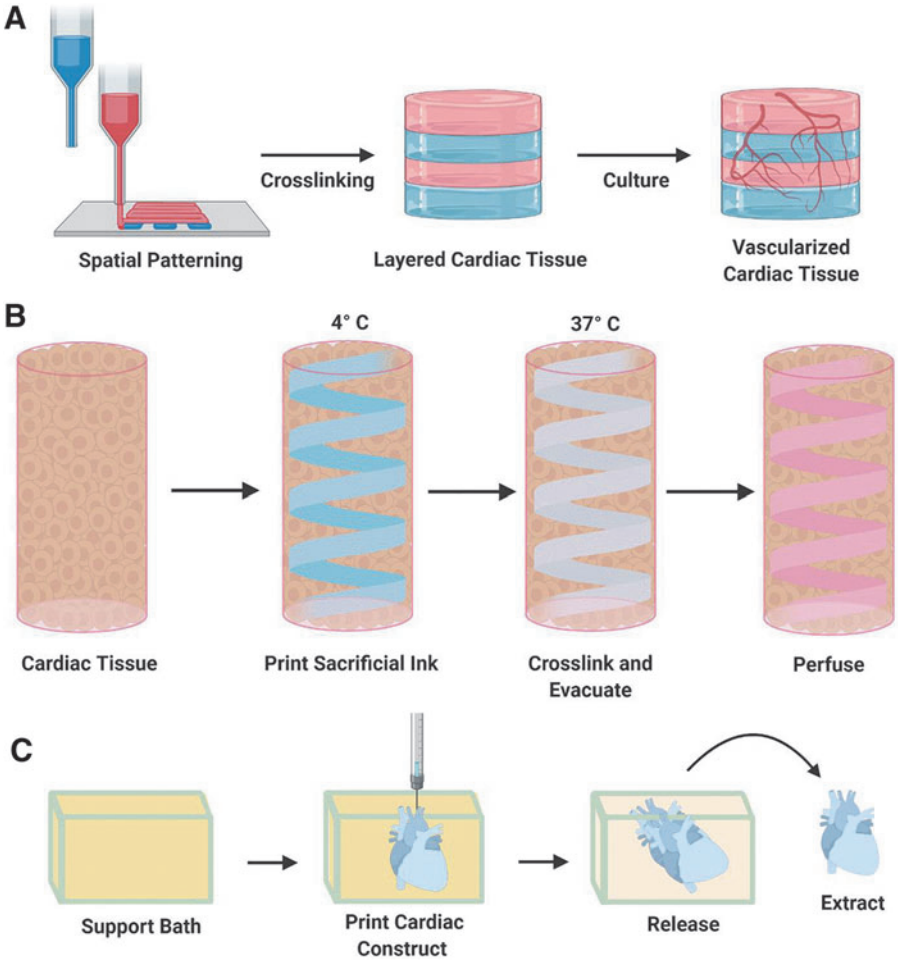


FIG. 4. 3D printing provides a platform for constructing detailed architectures of cardiac tissues **(A)** Bioinks composed of various cell types can be spatially patterned to give rise to functional cardiac tissues that can be subsequently evaluated both *in vitro* and *in vivo*. **(B)** Sacrificial inks are 3D printed into cardiac tissues and subsequently removed to create embedded luminal structures within engineered cardiac tissues that support thick tissue perfusion. **(C)** Support baths used 3D print soft materials with high fidelity that exhibit complex hierarchal structures of *in vivo* heart anatomy. 3D, three dimensional.

precisely control deposition of materials layer by layer. It provides unique advantages when reconstructing hierarchical human tissue structures composed of various types of cells. Thus, 3D printing has been used as a suitable method to engineer thick tissues with complex physiologically relevant architectures and/or organ-specific functionalities.[112–116] Many approaches have been proposed to mimic spatial patterning of organs with tissue-specific cell types, while simultaneously mimicking biological and mechanical properties of the tissue.[117] However, the integration of functional vasculatures remains to be one of the largest challenges when engineering thick tissues using 3D printing methods.[52] To this end, we discuss the most recent state-of-the-art advances made in 3D printing of vascularized cardiac tissues for the purpose of disease modeling and regenerative medicine.

Spatial patterning of cardiac and vascular tissues

Human organs and tissues have complex architectures in 3D that are necessary to fulfill their function. These architectures must be recapitulated in engineered tissues. For example, the use of multiple bioinks is a common strategy to construct the hierarchal structure of vascularized cardiac tissues. 3D bioprinters with incorporated microfluidic channels are capable of printing multiple bioinks both homogenously and heterogeneously with high fidelity (Fig. 4A).[118,119] For example, alginate bioinks can be crosslinked through electrostatic interactions[120] with Ca^{2+} so that coextrusion of the Ca^{2+} solution with bioink in a microfluidic system instantaneously forms hydrogel networks upon exiting the nozzle, ensuring structural stability during the printing process.[121] With multiple reservoirs incorporated into this microfluidic system, bioinks containing cardiac-specific cell types can be extruded either individually or simultaneously to achieve unique, spatially patterned hydrogels. The mechanical properties of the printed construct can be precisely tuned to the target tissue by incorporating ultra violet crosslinking sites, such as gelatin methacroyl.[122–124] Fine tuning of these properties for cardiac tissues and incorporating varying components (i.e., gold nanoparticles) can improve cardiac cell viability and electrical propagation, and promote overall functionality.[125] In a recent study, iPSC-CM were printed with EC in an alginate bioink infused with polyethylene glycol monoacrylate-fibrinogen (PF).[126] The mechanical properties of the printed construct were fine-tuned to the target tissue by modulating the UV crosslinking density of the infused PF. In addition, the alginate was partially removed using a chelating agent (ethylenediaminetetraacetic acid) to ensure sufficient diffusion of the culture media and improve overall cell viability.[127] They observed that the side-by-side coextrusion of the same numbers of iPSC-CM and HUVEC exhibited the highest degree of CM alignment

and viability for both cell types *in vitro.* Also, coextrusion patterning exhibited maximum recruitment of host vasculature into the cardiac patch in murine MI models.

3D printed sacrificial inks

In contrast to spatially patterning cardiac-specific cell types, some have looked toward using sacrificial inks, materials that are removable after the printing process, to directly imbed luminal structures into cardiac tissues (Fig. 4B). Sacrificial inks are printed in 3D at a desirable geometry together with tissue-specific, cell-laden biomaterials that can be subsequently crosslinked to support structural integrity. Following the removal of the sacrificial inks, open lumens remain and serve as physical conduits for EC to be seeded to form blood vessels within a thick tissue, enabling diffusive mass transport of nutrients, oxygen, and metabolic waste.[128–130] Carbohydrate glass is one of the common sacrificial inks used to form perfusable vascular networks in biomaterials.[131–133] Carbohydrate glass can be printed at an elevated temperature (110°C), but rapidly solidifies at room temperature leaving behind a mechanically stable structure.[131,134] Once established, the printed lattice can be coated with poly(D-lactide-co-glycolide) (PDLGA) to facilitate the clearance of carbohydrate glass through the printed network and prevent osmotic damage to cells within the bulk construct due to the lack of media transport.[131] Carbohydrate glass constructs can be cured within a wide variety of hydrogels, including agarose, alginate, PEG, fibrin, Matrigel, and collagen, among others.[131] This method was utilized to engineer a vascular cardiac patch through printing of a fibrin hydrogel with various luminal structures. This was transplanted into a region of infarct in a rat MI model, where it successfully recruited host vasculatures and induced a substantial increase in host capillary beds within the region of infarct. This served to increase the ejection fraction and cardiac output along with a decrease in left ventricular internal dimension.[62]

Thermosensitive bioinks are also commonly used to create sacrificial lumen structures in engineered tissues.[129,135–137] These bioinks undergo a thermally reversible gelation process as the temperature changes and can be dissolved in an aqueous solvent or evacuated from the tissue. This technology was utilized to print the major coronary arteries of the left ventricle in a cardiac patch in 3D (Fig. 5A–G).[138] To more closely mimic vascular patterns in the heart, they utilized computerized tomography (CT) to scan the 3D distribution of vasculatures *in vivo*, generate computer-aided designs (CAD), and print 3D tissue constructs accordingly in a layer-by-layer process. In a bottom-up approach, they printed EC-laden thermosensitive gelatin in parallel with decellularized omentum containing iPSC-CM to fabricate a vascularized cardiac patch. At physiological temperature, the Young's modulus of gelatin markedly drops, allowing it to be washed away, leaving open lumens embedded into the tissue.[139] Following 7 days in culture, lumens within the cardiac patch were lined with confluent monolayers of EC and the neighboring cardiac tissue was capable of depolarization. Therapeutic potential of the cardiac patches was also assessed *in vivo* when transplanted into rat omentum for a 7-day period, where it was observed that the CM were elongated and aligned, and had an exceptional degree of striation, demonstrating further maturation of the cardiac patch following implantation.

In a recent work, Skylar-Scott and Uzel *et al.* demonstrated a technique for 3D printing vasculature into iPSC-CM tissues using a thermally sacrificial gelatin ink, termed ''sacrificial writing into functional tissue'' (SWIFT) (Fig. 5H–L).[140] In their work, gelatin sacrificial inks were directly printed into a viscoelastic slurry of iPSC-CM organoids and engineered ECM. The gelatin sacrificial ink was printed at 2°C so that the sacrificial ink could maintain its mechanical stiffness at an order of magnitude higher than the ECM/CM organoid slurry. Once brought to physiological temperature, the thick ECM/CM organoid slurry crosslinks, while the gelatin sacrificial ink melts leaving behind large perfusable lumens. The vascularized cardiac tissue was able to beat synchronously over an 8-day period and showed increased overall cardiac displacement over time, indicating cardiac tissue maturation. In addition, the authors were able to demonstrate that SWIFT was capable of utilizing CT imaging-inspired CAD to 3D print the hierarchical architecture of the LAD within the ECM/CM slurry.

3D printing of cardiac structures in support materials

While sacrificial inks are used to provide a material that can be subsequently removed, allowing for the development of lumens in the printed tissue, some tissues additionally need more structural support during printing. This is due to the poor mechanical stability of many printable hydrogels and biomaterials of physiological stiffness. Sacrificial constructs have been printed within support materials, which allows for higher print resolution and fidelity of soft materials (Fig. 4C).[141–144] In one approach, sacrificial inks were printed into modified photocurable support gels to engineer omnidirectionally pervasive vascular networks.[129] Sacrificial inks printed into support gels composed of Pluronic F127 diacrylate generally maintain the shape and position at the point of extrusion. Because the support material easily deforms as the extrusion needle moves through it, creating a void in the gel, low viscosity Pluronic F127 diacrylate gel was poured in to fill voids created during the printing procedure. The resulting printed constructs were crosslinked with UV light and the sacrificial ink was removed, allowing for embedded vasculature within the tissue construct.

Building on this concept in a seminal work, Hinton *et al.* described a method allowing 3D printing of soft materials within a support bath termed freeform reversible embedding of suspended hydrogels (FRESH).[141] This methodology relies on the mechanical stability of gelatin at specific temperatures and the pH range at which collagen crosslinks. In this technique, a gelatin support bath acts as a Bingham plastic at room temperature, allowing a moving nozzle to flow through it, while depositing material without disrupting the structural integrity of the support bath. The collagen is kept in a highly acidic environment preventing crosslinking and allowing for extrusion from the print nozzle. Once extruded from the nozzle, the collagen is deposited into the gelatin bath kept at a neutral pH of 7.4 where the collagen rapidly crosslinks, leaving behind a solid structure within the gelatin. Subsequently, the construct and bath are warmed to 37°C, causing the gelatin to melt, while the printed collagen maintains structural integrity and is released by the

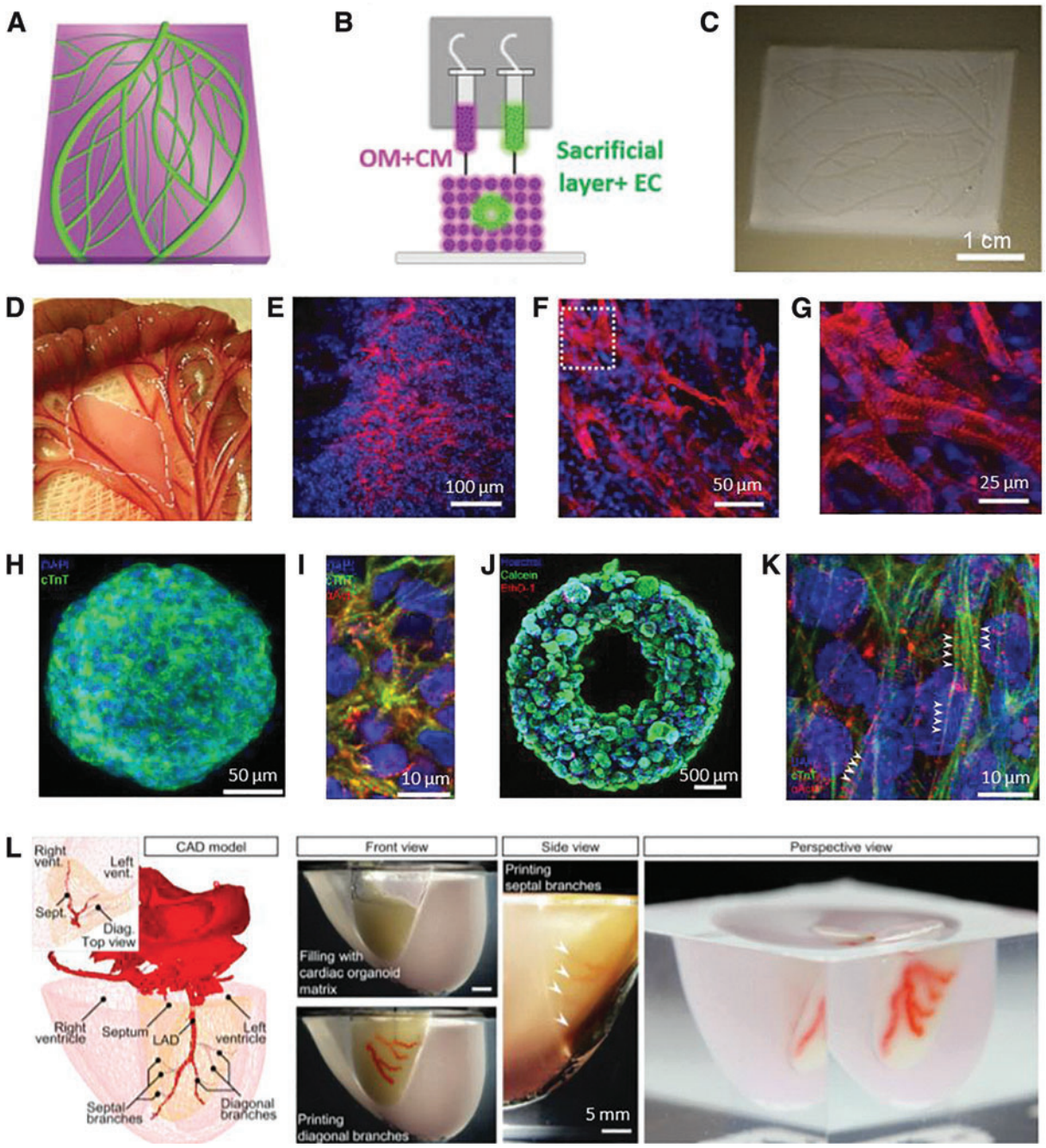


FIG. 5. 3D printing enables the generation of functional vascular cardiac tissues and complex cardiovascular structures. **(A–C)** Overview of bottom-up 3D printing process for the generation of a cardiac patch.[138] **(D)** Image of excised omentum following 7 days of *in vivo* transplantation with *white dashed lines* highlighting the border of the cardiac patch.[138] **(E–G)** Imaging of excised 3D printed cardiac patch following 7 days of *in vivo* transplantation for sarcomeric actinin (*red*) exhibiting a matured cardiac morphology at increasing magnification.[138] **(H, I)** Cardiac organoid organ building block staining positive for cardiac specific markers cardiac troponin T (*green*) and α-actinin (*red*).[140] **(J, K)** Viable SWIFT 3D printed perusable cardiac tissues imaged from top-down staining positive for cardiac troponin T and α-actinin following 24 h of perfusion.[140] **(L)** SWIFT printed CAD rendering of left anterior descending artery into left ventricle-shaped mold.[140] CAD, computer-aided designs; SWIFT, sacrificial writing into functional tissue.

support bath. In this study, the gelatin support bath incorporated irregularly shaped microparticles, which limited print resolution to ~65 µm. To overcome this obstacle in a more recent work, the group developed FRESH v2.0, which incorporates gelatin microparticles of ~25 µm diameter, improving the print resolution.[145] The resolution of the prints was showcased by printing CAD-rendered CT scans of high-resolution architectures of the heart, including tri-leaflet heart valves, LAD branching, and a full-sized neonatal-scale human heart containing features such as atrial and ventricular chambers, trabeculae, and pulmonary and aortic valves (Fig. 6A–E). In addition, the authors also

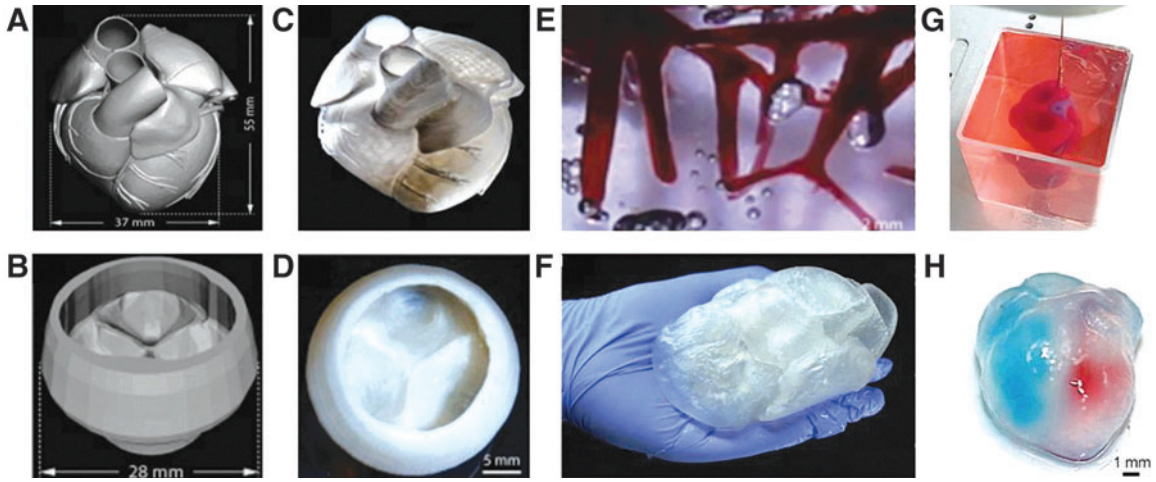


FIG. 6. 3D printing into support material **(A, B)** CAD-generated renderings of cardiac structures used for FRESH v2.0 3D printing.[145] **(C, D)** FRESH v2.0 printed neonatal scale human heart exhibiting complex cardiac morphology and adult size tri-leaflet heart valve, respectively.[145] **(E)** FRESH v2.0 printed construct based on MRI-derived CAD rendering of the left anterior descending artery with computationally generated microvasculature capable of perfusion.[145] **(F)** FRESH v2.0 printed full-size adult human heart.[146] **(G, H)** Heart containing independent chambers, vasculature, and imbedded CMs and ECs printed into alginate microparticle support bath.[138] FRESH, freeform reversible embedding of suspended hydrogels.

reported using FRESH v2.0 to print a scaffold of a ventricle that was populated with cardiac cells that exhibited directional wave propagation during spontaneous contraction, which maintained viability for up to 28 days. In a more recently published article, the group reported using FRESH v2.0 support baths to print a full-sized human heart composed of alginate based on MRI CAD renderings to train for and plan out cardiothoracic surgeries (Fig. 6F).[146] Other groups have also expanded on freeform reversible printing to afford engineered vascular cardiac tissues capable of both perfusion and depolarization.[144,147]

Support baths comprising alginate microparticles have also been used in lieu of gelatin.[138] This can be advantageous when printing hydrogels that crosslink at 37°C due to the fact that alginate will not melt at this temperature. The alginate support bath can be brought to 37°C, allowing for the printed hydrogel to crosslink in place and maintain shape within the support bath. Once printed, the alginate support bath can be dissolved through an enzymatic process rather than a thermal process. In a recent work, this method was used to print a model of the heart containing CM and EC.[138] CM were embedded into a dECM hydrogel, which were printed as cardiac walls with distinct chambers on both the left and right side of the heart (Fig. 6G, H). In addition, a major blood vessel was printed using EC embedded into a dECM hydrogel that wrapped around the periphery of the cardiac wall. Once printed, the bath was brought to 37°C allowing for crosslinking of the dECM hydrogel and subsequent enzymatic removal. It was reported that 1 day after printing, there was homogenous distribution of CM within the printed construct, but no further structural or functional findings were reported.

Conclusion and Perspective

In the rapidly evolving field of engineered cardiac tissues, there have been remarkable advances and discoveries made in both basic and applied sciences. Biomaterials are continually investigated, but biocompatibility of the materials is often compromised to provide improved structural integrity and vice versa. In addition, the ability to provide the correct physiochemical cues for all cell types native to the myocardium has yet to be fully addressed. While these challenges still need to be addressed, the greatest challenges in the field are finding ways to not only introduce vasculature but also to engineer pervasive capillary networks within functional cardiac tissues. Cardiac patches and cell sheets show potential in the ability to recruit host vasculature when transplanted *in vivo,* but their full integration with the host, abilities to synchronously contract with host myocardium, and functional restoration following MI are not yet suitable as clinical therapeutics. 3D printing shows great promise in the ability to pattern hierarchal architectures of the heart and their vasculature, but is ultimately hindered by the material properties of bioinks and the resolution of the print when printing connective capillary beds. Another limitation in the field is that the majority of engineered vascularized cardiac tissues use EC types not specific to cardiac tissue. The identification of signaling pathways that regulate cardiac-specific endothelial differentiation and expansion in culture would significantly contribute to advancing cell-based therapies for cardiac regeneration. It should also be noted that in addition to blood vessels, lymphatic vessels in the heart play a critical role in cardiac health and disease. The importance of lymphatic vasculature in cardiac development and repair is reviewed in detail by Klaourakis *et al.*[148] However, to our best knowledge, there have been no attempts to incorporate tissue-engineered lymphatic vessels into engineered cardiac tissues. Since lymphatic vessels help wound healing in the heart, preventing fibrosis, more complex models containing tissue-engineered lymphatic vessels in the vascularized cardiac tissues would be a strong improvement to existing constructs. As we further explore materials, exploit biological pathways, and refine methodologies, we can more closely study human cardiac disease pathology and eventually engineer functional, vascularized cardiac tissues as therapeutic implants in clinic.

Authors' Contributions

M.A.C.W. wrote main body of text and generated figures. D.M. wrote portions of text, edited, generated figures, and generated tables. E.L. wrote portions of text and provided editing. W.L. wrote portions of text and provided editing. D.-H.K. devised the main conceptual ideas and proof outline, oversaw writing, and provided editing.

Author Disclosure Statement

D.-H.K. is a scientific founder and equity holder of Curi Bio. The other authors report no conflicts.

Funding Information

This work was supported by grants from the National Institutes of Health for R01HL135143, R01HL146436, UG3TR003271, UH3TR003519 (to D.-H.K), R25GM109441 (to M.A.C.W.), and K25CA201545 (to W.L.). This work was also supported by the Cornell Nancy and Peter Meinig Family Investigator funds (to E.L.).

References

1. Savarese, G., and Lund, L.H. Gloabal public health burden of heart failure. J Hear Team **3,** 51, 2016.
2. Panza, J.A., Ellis, A.M., Al-Khalidi, H.R., *et al.* Myocardial viability and long-term outcomes in ischemic cardiomyopathy. N Engl J Med **381,** 739, 2019.
3. Nowbar, A.N., Gitto, M., Howard, J.P., Francis, D.P., and Al-Lamee, R. Mortality from ischemic heart disease: analysis of data from the world health organization and coronary artery disease risk factors from NCD risk factor collaboration. Circ Cardiovasc Qual Outcomes **12,** 1, 2019.
4. World Health Organization. Global Health Estimates 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000–2016.
5. Michler, R.E., Smith, P.K., Parides, M.K., *et al.* Two-year outcomes of surgical treatment of moderate ischemic mitral regurgitation. N Engl J Med **374,** 1932, 2016.
6. Ellison, D.H., and Felker, G.M. Diuretic treatment in heart failure. N Engl J Med **377,** 1964, 2017.
7. Marrouche, N.F., Brachmann, J., Andresen, D., *et al.* Catheter ablation for atrial fibrillation with heart failure. N Engl J Med **378,** 417, 2018.
8. Stone, G.W., Lindenfeld, J.A., Abraham, W.T., *et al.* Transcatheter mitral-valve repair in patients with heart failure. N Engl J Med **379,** 2307, 2018.
9. Køber, L., Thune, J.J., Nielsen, J.C., *et al.* Defibrillator implantation in patients with nonischemic systolic heart failure. N Engl J Med **375,** 1221, 2016.
10. Metra, M., Teerlink, J.R., Cotter, G., *et al.* Effects of serelaxin in patients with acute heart failure. N Engl J Med **381,** 716, 2019.
11. Abouna, G.M. Organ shortage crisis: problems and possible solutions. Transplant Proc **40,** 34, 2008.
12. Bajaj, P., Schweller, R.M., Khademhosseini, A., West, J.L., and Bashir, R. 3D Biofabrication strategies for tissue engineering and regenerative medicine. Annu Rev Biomed Eng **16,** 247, 2014.
13. Wu, J., Zheng, Z., Chong, Y., *et al.* Immune responsive release of tacrolimus to overcome organ transplant rejection. Adv Mater **30,** 1, 2018.
14. Nankivell, B.J., and Alexander, S.I. Rejection of the kidney allograft. N Engl J Med **364,** 485, 2011.
15. Loupy, A., and Lefaucheur, C. Antibody-mediated rejection of solid-organ allografts. N Engl J Med **379,** 1150, 2018.
16. Cohen-Mansfield, J., Dakheel-Ali, M., Marx, M.S., Thein, K., and Regier, N.G.P. Human iPSC-derived cardiomyocytes and tissue engineering strategies for disease modeling and drug screening. Physiol Behav **117,** 80, 2017.
17. Takahashi, K., and Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell **126,** 663, 2006.
18. Smith, A.S.T., Macadangdang, J., Leung, W., Laflamme, M.A., and Kim, D.H. Human iPSC-derived cardiomyocytes and tissue engineering strategies for disease modeling and drug screening. Biotechnol Adv **35,** 77, 2017.
19. Mandrycky, C.J., Williams, N.P., Batalov, I., *et al.* Engineering heart morphogenesis. Trends Biotechnol **38,** 835, 2020.
20. Wang, Z., Mithieux, S.M., and Weiss, A.S. Fabrication techniques for vascular and vascularized tissue engineering. Adv Healthc Mater **8,** 1, 2019.
21. Ramaswamy, A.K., Vorp, D.A., and Weinbaum, J.S. Functional vascular tissue engineering inspired by matricellular proteins. Front Cardiovasc Med **6,** 1, 2019.
22. Song, H.H.G., Rumma, R.T., Ozaki, C.K., Edelman, E.R., and Chen, C.S. Vascular tissue engineering: progress, challenges, and clinical promise. Cell Stem Cell **22,** 340, 2018.
23. Jain, R.K., Au, P., Tam, J., Duda, D.G., and Fukumura, D. Engineering vascularized tissue. Nat Biotechnol **23,** 821, 2005.
24. Kannan, R.Y., Salacinski, H.J., Sales, K., Butler, P., and Seifalian, A.M. The roles of tissue engineering and vascularisation in the development of micro-vascular networks: a review. Biomaterials **26,** 1857, 2005.
25. Turner, R., Beckstead, J., Warnke, R., and Wood, G. Endothelial cell phenotypic diversity: in situ demonstration of immunologic and enzymatic heterogeneity that correlates with specific morphologic subtypes. Am J Pathol **141,** 569, 1992.
26. Tirziu, D., Giordano, F., and Simons, M. Cell communications in the heart. Circulation **112,** 928, 2010.
27. Giacomelli, E., Bellin, M., Sala, L., *et al.* Three-dimensional cardiac microtissues composed of cardiomyocytes and endothelial cells co-differentiated from human pluripotent stem cells. Dev **144,** 1008, 2017.
28. Page, C., Rose, M., Yacoub, M., and Pigott, R. Antigenic heterogeneity of vascular endothelium. Am J Pathol **141,** 673, 1992.
29. Garry, D.J., and Olson, E.N. A common progenitor at the heart of development. Cell **127,** 1101, 2006.
30. Jeon, H., Tsui, J.H., Jang, S.I., *et al.* Combined effects of substrate topography and stiffness on endothelial cytokine and chemokine secretion. ACS Appl Mater Interfaces **7,** 4525, 2015.
31. Helle, E., Ampuja, M., Dainis, A., *et al.* Rved endothelial cells and cardiomyocytes. bioRxiv **21,** 1, 2020.
32. Coppiello, G., Collantes, M., Sirerol-Piquer, M.S., *et al.* Meox2/Tcf15 heterodimers program the heart capillary endothelium for cardiac fatty acid uptake. Circulation **131,** 815, 2015.
33. Lother, A., Bergemann, S., Deng, L., Moser, M., Bode, C., and Hein, L. Cardiac endothelial cell transcriptome. Arterioscler Thromb Vasc Biol **38,** 566, 2018.

34. Brutsaert, D.L. Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. Physiol Rev **83,** 59, 2003.
35. Segers, V.F.M., Brutsaert, D.L., and De Keulenaer, G.W. Cardiac remodeling: endothelial cells have more to say than just NO. Front Physiol **9,** 382, 2018.
36. Gu, L., and Mooney, D.J. Biomaterials and emerging anticancer therapeutics: Engineering the microenvironment. Nat Rev Cancer **16,** 56, 2016.
37. Abbott, R.D., and Kaplan, D.L. Strategies for improving the physiological relevance of human engineered tissue. Trends Biotechnol **132,** 1, 2011.
38. Ingber, D.E. Mechanobiology and diseases of mechanotransduction. Ann Med **35,** 564, 2003.
39. Nelson, C.M., VanDuijn, M.M., Inman, J.L., Fletcher, D.A., and Bissell, M.J. Tissue geometry determines sites of mammary branching morphogenesis in organotypic cultures. Science **314,** 298, 2006.
40. Lee, K., Silva, E.A., and Mooney, D.J. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. J R Soc Interface **8,** 153, 2011.
41. Kim, T.J., Lei, L., Seong, J., *et al.* Matrix rigidity-dependent regulation of Ca2+ at plasma membrane microdomains by FAK visualized by fluorescence resonance energy transfer. Adv Sci **6,** 2019.
42. Raja, G., Cao, S., Kim, D.H., and Kim, T.J. Mechanoregulation of titanium dioxide nanoparticles in cancer therapy. Mater Sci Eng C **107,** 110303, 2020.
43. Long, J., Kim, H., Kim, D., Lee, J.B., and Kim, D.H. A biomaterial approach to cell reprogramming and differentiation. J Mater Chem B **5,** 2375, 2017.
44. Kim, S.J., Tatman, P.D., Song, D.H., Gee, A.O., Kim, D.H., and Kim, S.J. Nanotopographic cues and stiffness control of tendon-derived stem cells from diverse conditions. Int J Nanomed **13,** 7217, 2018.
45. Bugg, D., Bretherton, R., Kim, P., *et al.* Infarct collagen topography regulates fibroblast fate via p38-yes-associated protein transcriptional enhanced associate domain signals. Circ Res **23**, 1306, 2020.
46. Kim, D.-H., Wong, K.W., Park, J., Levchenko, A., and Sun, Y. Microengineered platforms for cell mechanobiology. Annu Rev Biomed Eng **11,** 203, 2009.
47. Ruprecht, V., Monzo, P., Ravasio, A., *et al.* How cells respond to environmental cues—insights from bio-functionalized substrates. J Cell Sci **130,** 51, 2017.
48. Huang, H., Kamm, R.D., and Lee, R.T. Cell mechanics and mechanotransduction: pathways, probes, and physiology. Am J Physiol **287,** 1, 2004.
49. Briquez, P.S., Clegg, L.E., Martino, M.M., Gabhann, F.M., and Hubbell, J.A. Design principles for therapeutic angiogenic materials. Nat Rev Mater **1,** 1, 2016.
50. Liu, H., Wang, Y., Cui, K., Guo, Y., Zhang, X., and Qin, J. Advances in hydrogels in organoids and organs-on-a-chip. Adv Mater **31,** 1, 2019.
51. Kim, J.J., Hou, L., and Huang, N.F. Vascularization of three-dimensional engineered tissues for regenerative medicine applications. Acta Biomater **176,** 139, 2017.
52. Tomasina, C., Bodet, T., Mota, C., Moroni, L., and Camarero-Espinosa, S. Bioprinting vasculature: materials, cells and emergent techniques. Materials **12,** 2701, 2019.
53. Salehi, S., Koeck, K., and Scheibel, T. Spider silk for tissue engineering applications. Molecules **25,** 737, 2020.
54. Chawla, S., Midha, S., Sharma, A., and Ghosh, S. Silk-based bioinks for 3D bioprinting. Adv Healthc Mater **7,** 1, 2018.
55. Rose, J.C., and De Laporte, L. Hierarchical design of tissue regenerative constructs. Adv Healthc Mater **7,** 1, 2018.
56. Mu, X., Fitzpatrick, V., and Kaplan, D.L. From silk spinning to 3D printing: polymer manufacturing using directed hierarchical molecular assembly. Adv Healthc Mater **9,** 1, 2020.
57. Liaw, C.Y., Ji, S., and Guvendiren, M. Engineering 3D hydrogels for personalized in vitro human tissue models. Adv Healthc Mater **7,** 1, 2018.
58. Annabi, N., Tamayol, A., Uquillas, J.A., *et al.* 25th anniversary article: rational design and applications of hydrogels in regenerative medicine. Adv Mater **26,** 85, 2014.
59. Chung, J.J., Kanade, R., and Atluri, P. New and improved: implications of a cardiac support device composed of biodegradable materials. Semin Thorac Cardiovasc Surg **29,** 62, 2017.
60. Huang, G., Li, F., Zhao, X., *et al.* Functional and biomimetic materials for engineering of the three-dimensional cell microenvironment. Chem Rev **176,** 139, 2017.
61. Thakuri, P.S., Liu, C., Luker, G.D., and Tavana, H. Biomaterials-based approaches to tumor spheroid and organoid modeling. Adv Healthc Mater **176,** 139, 2017.
62. Kim, P., Yuan, A., Nam, K.-H., Jiao, A., and Kim, D.-H. Fabrication of poly(ethylene glycol): gelatin methacrylate composite nanostructures with tunable stiffness and degradation for vascular tissue engineering. Biofabrication **6,** 024112, 2014.
63. Greggio, C., De Franceschi, F., Figueiredo-Larsen, M., *et al.* Artificial three-dimensional niches deconstruct pancreas development in vitro. Development **140,** 4452, 2013.
64. Zhang, C., Hekmatfer, S., and Karuri, N.W. A comparative study of polyethylene glycol hydrogels derivatized with the RGD peptide and the cell-binding domain of fibronectin. J Biomed Mater Res **102,** 170, 2014.
65. Gjorevski, N., Sachs, N., Manfrin, A., *et al.* Designer matrices for intestinal stem cell and organoid culture. Nature **539,** 560, 2016.
66. Turturro, M.V., Christenson, M.C., Larson, J.C., Young, D.A., Brey, E.M., and Papavasiliou, G. MMP-sensitive PEG diacrylate hydrogels with spatial variations in matrix properties stimulate directional vascular sprout formation. PLoS One **8,** e58897, 2013.
67. Wang, Z., Lee, S.J., Cheng, H., Yoo, J.J., and Atala, A. 3D bioprinted functional and contractile cardiac tissue constructs. Acta Biomater **70,** 48, 2018.
68. Sun, A.X., Lin, H., Fritch, M.R., *et al.* Chondrogenesis of human bone marrow mesenchymal stem cells in 3-dimensional, photocrosslinked hydrogel constructs: effect of cell seeding density and material stiffness. Acta Biomater **176,** 139, 2017.
69. Wang, Z., Abdulla, R., Parker, B., Samanipour, R., Ghosh, S., and Kim, K. A simple and high-resolution stereolithography-based 3D bioprinting system using visible light crosslinkable bioinks. Biofabrication **7,** 045009, 2015.
70. Perry, L., Merdler, U., Elishaev, M., and Levenberg, S. Enhanced host neovascularization of prevascularized engineered muscle following transplantation into immunocompetent versus immunocompromised mice. Cells **8,** 1472, 2019.

71. Kiseleva, A.P., Krivoshapkin, P.V., and Krivoshapkina, E.F. Recent advances in development of functional spider silk-based hybrid materials. Front Chem **8,** 1, 2020.
72. Smith, A.S.T., Yoo, H., Yi, H., *et al.* Micro-and nanopatterned conductive graphene-PEG hybrid scaffolds for cardiac tissue engineering. Chem Commun **53,** 7412, 2017.
73. Smith, P.T., Narupai, B., Tsui, J.H., *et al.* Additive manufacturing of bovine serum albumin-based hydrogels and bioplastics. Biomacromolecules **21,** 484, 2020.
74. Tsui, J.H., Leonard, A., Camp, N.D., *et al.* Functional maturation of human iPSC-based cardiac microphysiological systems with tunable electroconductive decellularized extracellular matrices. bioRxiv 2019. DOI: 10.1101/786657.
75. Spiller, K.J.K.L., Anfang, R., Spiller, K.J.K.L., *et al.* Improved angiogenesis in response to localized delivery of macrophage-recruiting molecules. PLoS One **6,** 1, 2015.
76. Wang, F.Q., Chen, G., Zhu, J.Y., *et al.* M2-polarised macrophages in infantile haemangiomas: correlation with promoted angiogenesis. J Clin Pathol **66,** 1058, 2013.
77. Numasaki, M., Watanabe, M., Suzuki, T., *et al.* IL-17 enhances the net angiogenic activity and in vivo growth of human non-small cell lung cancer in SCID mice through promoting CXCR-2-dependent angiogenesis. J Immunol **175,** 6177, 2005.
78. Jetten, N., Verbruggen, S., Gijbels, M.J., Post, M.J., De Winther, M.P.J., and Donners, M.M.P.C. Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo. Angiogenesis **17,** 109, 2014.
79. Katabathina, V., Menias, C.O., Pickhardt, P., Lubner, M., and Prasad, S.R. Complications of immunosuppressive therapy in solid organ transplantation. Radiol Clin North Am **54,** 303, 2016.
80. Liau, B., Jackman, C.P., Li, Y., and Bursac, N. Developmental stage-dependent effects of cardiac fibroblasts on function of stem cell-derived engineered cardiac tissues. Sci Rep **7,** 1, 2017.
81. Ou, D.B., He, Y., Chen, R., *et al.* Three-dimensional coculture facilitates the differentiation of embryonic stem cells into mature cardiomyocytes. J Cell Biochem **112,** 3555, 2011.
82. Lesman, A., Habib, M., Caspi, O., *et al.* Transplantation of a tissue-engineered human vascularized cardiac muscle. Tissue Eng Part A **16,** 115, 2010.
83. Zamani, M., Karaca, E., and Huang, N.F. Multicellular interactions in 3D engineered myocardial tissue. Front Cardiovasc Med **5,** 1, 2018.
84. Li, Y., Asfour, H., Bursac, N., and Author, A.B. Age-dependent functional crosstalk between cardiac fibroblasts and cardiomyocytes in a 3D engineered cardiac tissue. Acta Biomater **55,** 120, 2017.
85. Ye, L., Chang, Y.H., Xiong, Q., *et al.* Cardiac repair in a porcine model of acute myocardial infarction with human induced pluripotent stem cell-derived cardiovascular cells. Cell Stem Cell **15,** 750, 2014.
86. Stevens, K.R., Kreutziger, K.L., Dupras, S.K., *et al.* Physiological function and transplantation of scaffold-free and vascularized human cardiac muscle tissue. Proc Natl Acad Sci U S A **106,** 16568, 2009.
87. Wendel, J.S., Ye, L., Tao, R., *et al.* Functional effects of a tissue-engineered cardiac patch from human induced pluripotent stem cell-derived cardiomyocytes in a rat infarct model. Stem Cells Transl Med **4,** 1324, 2015.
88. Gao, L., Gregorich, Z.R., Zhu, W., *et al.* Large cardiac muscle patches engineered from human induced-pluripotent stem cell-derived cardiac cells improve recovery from myocardial infarction in swine. Circulation **137,** 1712, 2018.
89. Michalopoulos, G.K. Liver regeneration. J Cell Physiol **2,** 286, 2007.
90. Carlson, B.M. Some principles of regeneration in mammalian systems. Anat Rec B New Anat **287,** 4, 2005.
91. Masuda, S., and Shimizu, T. Three-dimensional cardiac tissue fabrication based on cell sheet technology. Adv Drug Deliv Rev **96,** 103, 2016.
92. Sekine, H., Shimizu, T., Sakaguchi, K., *et al.* In vitro fabrication of functional three-dimensional tissues with perfusable blood vessels. Nat Commun **4,** 1, 2013.
93. Williams, N.P., Rhodehamel, M., Yan, C., *et al.* Engineering anisotropic 3D tubular tissues with flexible thermoresponsive nanofabricated substrates. Biomaterials **240,** 119856, 2020.
94. Mengsteab, P.Y., Uto, K., Smith, A.S.T., *et al.* Spatiotemporal control of cardiac anisotropy using dynamic nanotopographic cues. Biomaterials **86,** 1, 2016.
95. Jiao, A., Trosper, N.E., Yang, H.S., *et al.* Thermoresponsive nanofabricated substratum for the engineering of three-dimensional tissues with layer-by-layer architectural control. ACS Nano **8,** 4430, 2014.
96. Shimizu, T., Yamato, M., Isoi, Y., *et al.* Fabrication of pulsatile cardiac tissue grafts using a novel 3-dimensional cell sheet manipulation technique and temperature-responsive cell culture surfaces. Circ Res **90,** 1, 2002.
97. Sekiya, S., Shimizu, T., Yamato, M., Kikuchi, A., and Okano, T. Bioengineered cardiac cell sheet grafts have intrinsic angiogenic potential. Biochem Biophys Res Commun **341,** 573, 2006.
98. Shimizu, T., Sekine, H., Isoi, Y., Yamato, M., Kikuchi, A., and Okano, T. Long-term survival and growth of pulsatile myocardial tissue grafts engineered by the layering of cardiomyocyte sheets. Tissue Eng **12,** 499, 2006.
99. Sekine, H., Shimizu, T., Hobo, K., *et al.* Endothelial cell coculture within tissue-engineered cardiomyocyte sheets enhances neovascularization and improves cardiac function of ischemic hearts. Circulation **118,** 145, 2008.
100. Ishigami, M., Masumoto, H., Ikuno, T., *et al.* Human iPS cell-derived cardiac tissue sheets for functional restoration of infarcted porcine hearts. PLoS One **13,** 1, 2018.
101. Majesky, M.W. Vascular development. Arterioscler Thromb Vasc Biol **176,** 139, 2017.
102. Senger, D.R., and Davis, G.E. Angiogenesis. Cold Spring Harb Perspect Biol **3,** 1, 2011.
103. Chen, R.R., Silva, E.A., Yuen, W.W., and Mooney, D.J. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. Pharm Res **24,** 258, 2007.
104. Zhang, J., Zhu, W., Radisic, M., and Vunjak-Novakovic, G. Can we engineer a human cardiac patch for therapy? Circ Res **123,** 244, 2018.
105. Kohara, H., Tajima, S., Yamamoto, M., and Tabata, Y. Angiogenesis induced by controlled release of neuropeptide substance P. Biomaterials **31,** 8617, 2010.
106. Dvir, T., Kedem, A., Ruvinov, E., *et al.* Prevascularization of cardiac patch on the omentum improves its therapeutic outcome. Proc Natl Acad Sci U S A **106,** 14990, 2009.

107. Benameur, N., Caiani, E.G., Arous, Y., Ben Abdallah, N., and Kraiem, T. Parametric imaging for the assessment of cardiac motion: a review. Cardiovasc Eng Technol **9,** 377, 2018.
108. Migrino, R., Young, J., Ellis, S., *et al.* End-systolic volume index at 90 to 180 minutes into reperfusion therapy for acute myocardial infarction is a strong predictor of early and late mortality. The Global Utilization of Streptokinase and t-PA for Occluded Coronary Arteries (GUSTO)-I Angiograph. Circulation **96,** 116, 1997.
109. Marsano, A., Maidhof, R., Luo, J., *et al.* The effect of controlled expression of VEGF by transduced myoblasts in a cardiac patch on vascularization in a mouse model of myocardial infarction. Biomaterials **34,** 393, 2014.
110. Shafiq, M., Zhang, Y., Zhu, D., *et al.* In situ cardiac regeneration by using neuropeptide substance P and IGF-1C peptide eluting heart patches. Regen Biomater **5,** 303, 2018.
111. Tsao, C.J., Pandolfi, L., Wang, X., *et al.* Electrospun patch functionalized with nanoparticles allows for spatiotemporal release of VEGF and PDGF-BB promoting in vivo neovascularization. ACS Appl Mater Interfaces **10,** 44344, 2018.
112. Murphy, S.V., and Atala, A. 3D bioprinting of tissues and organs. Nat Biotechnol **32,** 773, 2014.
113. Pati, F., Jang, J., Ha, D.H., *et al.* Printing three-dimensional tissue analogues with decellularized extracellular matrix bioink. Nat Commun **5,** 3935, 2014.
114. Mandrycky, C., Wang, Z., Kim, K., and Kim, D.H. 3D bioprinting for engineering complex tissues. Biotechnol Adv **34,** 422, 2016.
115. Kim, H.N., Habbit, N.L., Su, C., *et al.* 3D bioprinting: microphysiological systems as enabling tools for modeling complexity in the tumor microenvironment and accelerating cancer drug development. Adv Funct Mater **29,** 1970146, 2019.
116. Shin, Y.J., Shafranek, R.T., Tsui, J.H., Walcott, J., Nelson, A., and Kim, D.H. 3D bioprinting of mechanically tuned bioinks derived from cardiac decellularized extracellular matrix. Acta Biomater **119,** 75, 2021.
117. Gungor-Ozkerim, P.S., Inci, I., Zhang, Y.S., Khademhosseini, A., and Dokmeci, M.R. Bioinks for 3D bioprinting: an overview. Biomater Sci **6,** 915, 2018.
118. Colosi, C., Shin, S.R., Manoharan, V., *et al.* Microfluidic bioprinting of heterogeneous 3D tissue constructs using low viscosity bioink. Adv Mater **28,** 677, 2016.
119. Jia, W., Gungor-ozkerim, P.S., Zhang, Y.S., *et al.* Direct 3D bioprinting of perfusable vascular constructs using a blend bioink. Biomaterials **106,** 58, 2016.
120. Kuo, C.K., and Ma, P.X. Ionically crosslinked alginate hydrogels as scafolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. Biomaterials **22,** 511, 2001.
121. Akbari, M., Tamayol, A., Laforte, V., *et al.* Composite living fibers for creating tissue constructs using textile techniques. Adv Funct Mater **24,** 4060, 2015.
122. Yue, K., Trujillo-de Santiago, G., Alvarez, M.M., Tamayol, A., Annabi, N., and Khademhosseini, A. Synthesis, properties, and biomedical applications of gelatin methacryloyl (GelMA) hydrogels. Biomaterials **73,** 254, 2015.
123. Pepelanova, I., Kruppa, K., Scheper, T., and Lavrentieva, A. Gelatin-methacryloyl (GelMA) hydrogels with defined degree of functionalization as a versatile toolkit for 3D cell culture and extrusion bioprinting. Bioengineering **5,** 55, 2018.
124. Zhu, M., Wang, Y., Ferracci, G., Zheng, J., Cho, N.J., and Lee, B.H. Gelatin methacryloyl and its hydrogels with an exceptional degree of controllability and batch-to-batch consistency. Sci Rep **9,** 1, 2019.
125. Zhu, K., Shin, S.R., Kempen, TV, *et al.* Gold nanocomposite bioink for printing 3D cardiac constructs. Adv Funct Mater **27,** 1605352, 2017.
126. Maiullari, F., Costantini, M., Milan, M., *et al.* A multicellular 3D bioprinting approach for vascularized heart tissue engineering based on HUVECs and iPSC-derived cardiomyocytes. Sci Rep **8,** 1, 2018.
127. Schulz, A., Gepp, M.M., Stracke, F., Briesen, H. Von, and Neubauer, J.C. Tyramine-conjugated alginate hydrogels as a platform for bioactive scaffolds. J Biomed Mater Res **107,** 114, 2019.
128. Gao, G., Park, J.Y., Kim, B.S., Jang, J., and Cho, D.W. Coaxial cell printing of freestanding, perfusable, and functional in vitro vascular models for recapitulation of native vascular endothelium pathophysiology. Adv Healthc Mater **7,** 1, 2018.
129. Wu, W., Deconinck, A., and Lewis, J.A. Omnidirectional printing of 3D microvascular networks. Adv Mater **23,** 178, 2011.
130. Ji, S., Almeida, E., and Guvendiren, M. 3D bioprinting of complex channels within cell-laden hydrogels. Acta Biomater **95,** 214, 2019.
131. Miller, J.S., Stevens, K.R., Yang, M.T., *et al.* Rapid casting of patterned vascular networks for perfusable engineered 3D tissues. Nat Mater **11,** 768, 2012.
132. Kinstlinger, I.S., Saxton, S.H., Calderon, G.A., *et al.* Generation of model tissues with dendritic vascular networks via sacrificial laser-sintered carbohydrate templates. Nat Biomed Eng **4,** 916, 2020.
133. Gauvin-Rossignol, G., Legros, P., Ruel, J., Fortin, M.A., and Bégin-Drolet, A. Sugar glass fugitive ink loaded with calcium chloride for the rapid casting of alginate scaffold designs. Heliyon **4,** e00680, 2018.
134. Reinheimer, M.A., Mussati, S., and Scenna, N.J. Influence of product composition and operating conditions on the unsteady behavior of hard candy cooling process. J Food Eng **101,** 409, 2010.
135. Daly, A.C., Pitacco, P., Nulty, J., Cunniffe, G.M., and Kelly, D.J. 3D printed microchannel networks to direct vascularisation during endochondral bone repair. Biomaterials **162,** 34, 2018.
136. Shao, L., Gao, Q., Xie, C., Fu, J., Xiang, M., and He, Y. Synchronous 3D bioprinting of large-scale cell-laden constructs with nutrient networks. Adv Healthc Mater **9,** 1, 2020.
137. Kolesky, D.B., Homan, K.A., Skylar-Scott, M.A., and Lewis, J.A. Three-dimensional bioprinting of thick vascularized tissues. Proc Natl Acad Sci U S A **113,** 3179, 2016.
138. Noor, N., Shapira, A., Edri, R., Gal, I., Wertheim, L., and Dvir, T. 3D printing of personalized thick and perfusable cardiac patches and hearts. Adv Sci **6,** 1900344, 2019.
139. Wen, H., Li, J., Payne, G.F., *et al.* Hierarchical patterning via dynamic sacrificial printing of stimuli-responsive hydrogels. Biofabrication **12,** 035007, 2020.
140. Skylar-Scott, M.A., Uzel, S.G.M., Nam, L.L., *et al.* Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels. Sci Adv **5,** eaaw2459, 2019.

141. Hinton, T.J., Jallerat, Q., Palchesko, R.N., *et al.* Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. Sci Adv **1,** e1500758, 2015.
142. Bhattacharjee, T., Zehnder, S.M., Rowe, K.G., *et al.* Writing in the granular gel medium. Sci Adv **1,** 4, 2015.
143. Hinton, T.J., Hudson, A., Pusch, K., Lee, A., and Feinberg, A.W. 3D printing PDMS elastomer in a hydrophilic support bath via freeform reversible embedding. ACS Biomater Sci Eng **2,** 1781, 2016.
144. Kupfer, M.E., Lin, W.H., Ravikumar, V., *et al.* In situ expansion, differentiation, and electromechanical coupling of human cardiac muscle in a 3D bioprinted, chambered organoid. Circ Res **127,** 207, 2020.
145. Lee, A., Hudson, A.R., Shiwarski, D.J., *et al.* 3D bioprinting of collagen to rebuild components of the human heart. Science **365,** 482, 2019.
146. Mirdamadi, E., Tashman, J.W., Shiwarski, D.J., Palchesko, R.N., and Feinberg, A.W. FRESH 3D bioprinting a full-size model of the human heart. ACS Biomater Sci Eng **6,** 6453, 2020.
147. Savoji, H., Davenport Huyer, L., Mohammadi, M.H., *et al.* 3D printing of vascular tubes using bioelastomer prepolymers by freeform reversible embedding. ACS Biomater Sci Eng **6,** 1333, 2020.
148. Klaourakis, K., Vieira, J.M., and Riley, P.R. The evolving cardiac lymphatic vasculature in development, repair and regeneration. Nat Rev Cardiol 2021 [Epub ahead of print]; DOI: 10.1038/s41569-020-00489-x.
149. Raica, M., and Cimpean, A.M. Platelet-derived growth factor (PDGF)/PDGF receptors (PDGFR) axis as target for antitumor and antiangiogenic therapy. Pharmaceuticals **3,** 572, 2010.
150. Jacobo, S.M.P., and Kazlauskas, A. Insulin-like growth factor 1 (IGF-1) stabilizes nascent blood vessels. J Biol Chem **290,** 6349, 2015.
151. Cross, M.J., and Claesson-Welsh, L. FGF and VEGF function in angiogenesis: Signalling pathways, biological responses and therapeutic inhibition. Trends Pharmacol Sci **22,** 201, 2001.
152. Hong, K.H., Ryu, J., and Han, K.H. Monocyte chemoattractant protein-1-induced angiogenesis is mediated by vascular endothelial growth factor-A. Blood **105,** 1405, 2005.
153. Montesano, R., and Orci, L. Tumor-promoting phorbol esters induce angiogenesis in vitro. Cell **42,** 469, 1985.
154. Hong, H.S., Lee, J., Lee, E., *et al.* A new role of substance P as an injury-inducible messenger for mobilization of $CD29^{+}$ stromal-like cells. Nat Med **15,** 425, 2009.
155. Sattler, K., and Levkau, B. Sphingosine-1-phosphate as a mediator of high-density lipoprotein effects in cardiovascular protection. Cardiovasc Res **82,** 201, 2009.

Address correspondence to:
Deok-Ho Kim, PhD
Department of Biomedical Engineering
Johns Hopkins University
724 Ross Research Building, 720 Rutland Avenue
Baltimore, MD 21205
USA

E-mail: dhkim@jhu.edu

Received: November 10, 2020
Accepted: February 8, 2021
Online Publication Date: March 16, 2021