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Establishing Early Functional Perfusion and Structure in Tissue Engineered Cardiac Constructs

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Abstract

Myocardial infarction (MI) causes massive heart muscle death and remains a leading cause of death in the world. Cardiac tissue engineering aims to replace the infarcted tissues with functional engineered heart muscles or revitalize the infarcted heart by delivering cells, bioactive factors, and/or biomaterials. One major challenge of cardiac tissue engineering and regeneration is the establishment of functional perfusion and structure to achieve timely angiogenesis and effective vascularization, which are essential to the survival of thick implants and the integration of repaired tissue with host heart. In this paper, we review four major approaches to promoting angiogenesis and vascularization in cardiac tissue engineering and regeneration: delivery of pro-angiogenic factors/molecules, direct cell implantation/cell sheet grafting, fabrication of prevascularized cardiac constructs, and the use of bioreactors to promote angiogenesis and vascularization. We further provide a detailed review and discussion on the early perfusion design in nature-derived biomaterials, synthetic biodegradable polymers, tissue-derived acellular scaffolds/whole hearts, and hydrogel derived from extracellular matrix. A better understanding of the current approaches and their advantages, limitations, and hurdles could be useful for developing better materials for future clinical applications.

Keywords

myocardial infarction; cardiac tissue engineering and regeneration; functional perfusion; angiogenesis; vascularization; cardiac constructs; biomaterials; biodegradable polymers; acellular scaffolds; whole organ decellularization; bioreactor

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I. INTRODUCTION

Acute myocardial infarction (MI) is caused by interruption of blood supply to the heart muscle as a severe outcome of coronary artery disease.⁶ After MI occurs, adult cardiomyocytes have limited self-healing ability to repair the diseased muscle, and current medical treatments also cannot effectively replace the necrotic or scarred cardiac tissues.⁷⁻⁹ Recently, cardiac regenerative medicine has come to the forefront as a new strategy for MI treatment, including stem cell injection, intramyocardial gene transfer, and cardiac tissue engineering,¹⁰⁻¹² with a goal to repair the infarcted tissues with the regenerated, functional cardiac muscle. The ambitious goal of cardiac tissue engineering is to create a functional cardiac construct that is able to (1) provide optimal structural, mechanical, and electrophysiological properties; (2) fully integrate into the native myocardium and synchronously beat with the neighboring cardiac muscle; and (3) stimulate the formation of vasculature networks for oxygen and nutrient supply.¹³

Up to now, various tissue constructs fabricated through tissue engineering approaches have been investigated or used in clinical settings as tissue patches or bioartificial scaffolds.¹⁴⁻¹⁶ However, a number of challenges still exist for constructing a fully functional complex tissue and organ, even though the *in vitro* engineering of tissues and organs, such as heart, lung, kidney, and liver, have been progressing. One of the major challenges is the thick tissue fabrication, which requires efficient recellularization of a thick tissue scaffold and the promotion of rapid angiogenesis and effective vasculature network in the constructs.

To overcome the challenge of thick tissue fabrication, the classic solution is to construct the prevascularized tissue for sufficient oxygen and nutrient delivery to improve the survival and function of the implanted engineered cardiac construct. The major approaches to promoting angiogenesis and vascularization in cardiac tissue engineering/regeneration include (1) delivery of pro-angiogenic molecules, such as growth factors, encoded genes, or cytokines to establish a vascular network within the damaged tissue; (2) direct cell implantation or cell sheet technology for cardiac tissue engineering; (3) fabrication of the prevascularized cardiac constructs prior to implantation; and (4) the use of bioreactors to promote angiogenesis and vascularization (Figure 1).

II. ANGIOGENESIS VIA BIOFACTORS/GROWTH FACTORS

Angiogenic growth factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF), have been demonstrated to play essential roles in the angiogenic process and the initial phase of angiogenesis.¹⁷⁻²⁰ One straightforward strategy is to inject the proangiogenic factors or appropriate encoding gene directly into the MI site. Several preclinical studies have demonstrated that pro-angiogenic factors, such as FGF-1, FGF-2, and VEGF, or encoding gene aid in reestablishing the blood flow and stimulate neovascularization within the ischemic myocardium regions.^{18,19,21-25} However, the study outcomes have shown limited success in clinically defined benefits, including survival, improvements in quality of life, and relief of symptoms.^{19,26} The unsatisfactory clinical outcomes might be due to lack of rapid angiogenesis and long-term maturation of the vascular network, in which the growth and

remodeling of the new vascular network fail to follow the hierarchical branching pattern seen with the normal vascular network.^{19,27}

Alternative approaches have focused on the controlled delivery of two or more types of growth factors, such as the co-delivery of bFGF and PDGF²⁸ and the combined delivery of bFGF, VEGFA, and VEGFC.²⁹ Other options include binding heparin to various angiogenic growth factors such as VEGF, bFGF, and TGF- β ^{30–32} designing controlled release system using heparin-conjugated natural polysaccharides,^{33–35} or synthesizing heparin-associated biodegradable materials to achieve local and sustained delivery of angiogenic growth factors such as bFGF.^{36,37} Although delivery of pro-angiogenic factors and molecules represents an attractive approach to promoting vascularization in MI treatment, there are still concerns on dosage control, short half-life *in vivo*, delivery methods, and limited potential in improving cardiac function.^{19,27,38–40}

III. CELL INJECTION AND CELL SHEET GRAFTING

A. Cell Injection

Cell based strategies for cardiac tissue engineering and revascularization have been investigated increasingly during the past years. For *in situ* cellular transplantation therapy, new cells are injected directly into the infarction region to replace the damaged cells and improve the cardiac function.^{41–43} Various cell types, including mesenchymal stem cells (MSCs), umbilical stem cells (USCs), embryonic stem cells (ESCs), cardiac stem cells, induced pluripotent cells (iPSCs), fetal cardiomyocytes, and skeletal myoblasts, have been investigated as cell sources for cellular transplantation therapy (Table 1).^{3,44–47} Certain types of stem cells, such as MSCs, ESCs, and iPSCs, have the potential to differentiate into multiple cell types and support angiogenesis, myogenesis, or both.^{18,19,48} The hope is that, after those cells are delivered into infarcted regions, they can help reestablish the vascular structure as well as support the growth and remodeling of new blood vessels. However, cell therapy has limited success because of the difficulties in controlling the size, location, and fate of the implanted cells. Another challenge for cell therapy is the lack of an appropriate extracellular environment to assist the retention of transplanted cells and the structural and biomechanical integration of the repaired region with the host tissue.^{49,50}

B. Cell Sheet Grafting

Cell sheet based technology stacks two-dimensional (2D) monolayer cell sheets into a multilayered, three-dimensional (3D) construct. The advantage of this technique is that it allows delivery of a large number of cells with retained cell-cell contact and possibly with cell-deposited ECM. Cell sheet grafting has the potential to dramatically improve the cell delivery efficiency and enhance the engraftment to host tissue after transplantation.^{51–55} Two techniques have been widely applied in creating the 3D cell sheet construct. The first is to manipulate a scaffold-free cell sheet directly, by stacking cell monolayers.^{51–53} The second method is to stack the single-layered cells using a supporting scaffold or membrane such as thermosensitive hydrogel.^{54,55}

Shimizu et al.⁵⁶ created a multilayered cardiomyocyte sheet graft and transplanted it subcutaneously into athymic rats. The microvasculature structure was found to form within a few days after transplantation, and long-term survival and pulsatile contractility were also confirmed.⁵⁶ An MSC sheet was also created and implanted onto the epicardial surface of the infarcted myocardium; the grafting subsequently improved ventricular wall thickness, cardiomyocyte differentiation, and vascular structure reconstruction.^{57–60} To improve the vascularization in the multilayered cell sheet construct, endothelial cells and cardiomyocytes have been mixed and co-cultured to yield a better prevascularized cellular construct.⁶¹ Interestingly, animal studies showed that the vascular network in the multilayered cell sheet construct had successfully connected with the host vessels.⁶¹ Nevertheless, the cell sheet technology still faces challenges before it can be introduced to clinical practice. Those challenges include the inefficient cell sheet harvesting and layering method, mechanical fragility, limited thickness of the cell sheet construct, insufficient vascularization before implantation, and slow vascular network remodeling post-surgery for efficient nutrient diffusion and oxygenation.^{62,63}

IV. CURRENT ATTEMPTS IN ESTABLISHING EARLY FUNCTIONAL PERFUSION IN CARDIAC CONSTRUCTS

A. Tissue Engineered Cardiac Muscles and Scaffolding

Cardiac muscles are highly defined, 3D organized, anisotropic tissues that make up the heart walls and enable propagation of electrical signals to produce coordinated mechanical contractions.⁶⁴ The multilayered helical architecture of the heart muscle is mediated by 3D myocardial ECM, which is an intriguing network composed of collagen (types I and III) fiber network, elastin, proteoglycans, and glycosaminoglycans (GAGs).^{65–68} The myocardial ECM network provides important mechanical functions in maintaining structural integrity, tethering myocytes, mediating myocyte contraction and relaxation, and preventing excessive stretching.⁶⁹ In addition to the structural ECM, cardiac muscles have an abundant blood supply supported by an extensive vascular network that nourishes the whole heart.⁷⁰ The cardiac muscles also have a much richer supply of mitochondria than the skeletal muscles, hence causing them to have a greater dependence on cellular respiration for adenosine triphosphate (ATP).⁷⁰

Cardiac tissue engineering aims to create functional tissue constructs with the right thickness, tissue compactness, proper density of functional cardiac cells, and ability to contract synchronously with the host heart muscle and restore cardiac function.⁶⁴ As we know, the survival of the thick tissue/organ construct relies on its access to the blood supply for oxygen and nutrients. To support survival and enhance functionality of cells in the inner core of the myocardial implant, tissue engineered cardiac construct needs to be either prevascularized or able to establish functional vasculature quickly after transplantation.^{71,72} Compared to the scaffold-free method, a tissue engineered cardiac construct has the potential to form capillary network via co-culturing cells (e.g., skeletal myoblasts, endothelial cells, endothelial progenitor cells, and embryonic fibroblasts) into the biocompatible porous scaffolds.^{71,72} The biocompatible porous structure can provide a

platform to allow the host vascular cell ingrowth and migration into the implanted construct, potentially inducing new vascular bed remodeling.^{73,74}

Therefore, one important factor for cardiac tissue engineering is to identify the ideal scaffold materials with proper biochemical and biophysical cues, which not only promote cardiac differentiation but also assist angiogenesis and vascularization.⁷⁵ The ideal cardiac scaffold should be biocompatible for both vascular cell types and other tissue-specific cell types, have controllable degradation profile, have mechanical properties matching native ECM, be permeable to nutrients and metabolic wastes, and have an oriented porous structure for cell migration and connection.⁷⁵ Eventually, the prevascularizable cardiac construct should be able to provide a hierarchical vascular network for blood circulation and supply nutrients for cells in the thick transplanted scaffold.^{71,72,76–78} In the following sections, we summarize the current available biomaterials utilized in cardiac tissue engineering, which can be categorized as (1) nature-derived biomaterials such as collagen, hyaluronic acid (HA), and alginate; (2) synthetic polymers such as polyglycolic acid (PGA), polylactic acid (PLA), and poly(lactic-co-glycolic acid) (PLGA); (3) tissue-derived acellular scaffolds such as decellularized myocardium and acellular urinary bladder-derived ECM; and (4) ECM-derived hydrogel.

B. Early Perfusion Design in Nature-Derived Biomaterials

Nature-derived biomaterials, such as collagen, fibronectin, alginate, and hyaluronic acid, are used as the platform for physiologic cell culture model and tissue engineering/regeneration. There are advantages and disadvantages associated with different nature-derived biomaterials. Collagen has negligible inflammatory and antigenic responses and is often employed as a cell culture substrate.⁷⁹ Hyaluronic acid mainly serves as the inert molecular filling for cartilage, skin, synovial fluid, and umbilical cords, and it also mediates other biological activities such as cell proliferation, morphogenesis, inflammation, and wound healing.⁸⁰ Alginate has been used for drug delivery, wound dressings, and cell culture.⁸¹

In cardiac tissue engineering, Tulloch et al.⁸² created the collagen based 3D myocardium with hESC-derived and hiPSC-derived cardiomyocytes, along with human endothelial cells under different regimens of mechanical conditioning. They found that the mechanical conditioning increased cardiomyocyte proliferation, hypertrophy, and alignment, whereas co-culturing with vascular and stromal cells further improved the vascular organization within the cardiac construct.⁸² Other studies also created a prevascularized 3D porous gelatin scaffold (denatured collagen) and cellularized this scaffold with hMSCs and human cardiomyocyte progenitor cells (hCMPCs).⁸³ Under continuous perfusion culture conditions, a densely packaged construct composed of vascular-like and cardiac-like cells was achieved.⁸³ Approaches in vascular tissue engineering might also benefit cardiac tissue engineering. For example, after mixing the pro-angiogenic factors, such as VEGF and FGF, into the ECM protein-derived gel, the endothelial cells seeded into the scaffold formed dense and durable vessels.^{84,85} Disadvantages of these nature-derived biomaterials are their lack of the tissue-specific architecture essential for cellular remodeling, weak mechanical properties, and rapid degradation kinetics, which must be overcome to achieve clinical applications.⁸⁶

C. Early Perfusion Design in Synthetic Biodegradable Polymers

Synthetic biodegradable polymers, such as PGA, poly(L-lactide) (PLLA), polyurethane, polycaprolactone (PCL), poly(glycerol sebacate), poly(caprolactone-co-lactide), polydioxanone, and poly(hydroxybutyrate), have been used as scaffold materials.^{87–94} These biodegradable polymeric materials are designed to mimic numerous physical environments to provide mechanical cues and cell signaling stimuli for assisting cell ingrowth and tissue remodeling. By altering the copolymer ratio and fabrication procedures, one can produce scaffolds with the controllable physical and chemical properties, tailored biodegradable profile, and well-characterized compositional and 3D structural properties to suit the tissue type it will be used to repair.^{87,95,96}

To mimic the vasculature networks inside the myocardial tissue, some synthetic scaffolds are designed to incorporate branching structures, microchannels, or interconnected pores.⁹⁷ The techniques to realize those structures include laser micro-fabrication,⁹⁸ patterning the microfluidic channels,⁹⁹ and 3D printing.¹⁰⁰ These approaches allow immediate perfusion via the vascular networks, assist in transfer of the nutrients, and promote cellular migration, attachment, and growth,^{101–103} with the potential to create “vascularized” 3D architecture of any shape and size. Marsano et al.¹⁰⁴ seeded a porous poly(glycerol sebacate) scaffold with cardiomyocytes, skeletal myoblasts, and VEGF and tested this construct using a mouse MI model. Their results showed the formation of the mature vascular network with a random arrangement inside the repaired infarcted tissue.¹⁰⁴ Lesman et al.^{105,106} successfully create a beating and prevascularized myocardium by recellularizing a porous scaffold made of 50% poly-L-lactic acid (PLLA) and 50% PLGA with a mixture of human ESCs differentiated into cardiomyocytes, fibroblasts, and endothelial cells (ECs). Along with the controlled release of^{105,106} VEGF, the scaffold fabricated from the mixture of synthetic polymers and hydrogels also showed successful vascular network formation and increased angiogenesis.¹⁰⁷ Still, the synthetic polymeric approach is facing issues such as inflammatory response, absence of biological signals, mismatched material properties, nonpliability, and difficulty in degradation profile control.^{92,108} It is worthwhile to note that, if not controlled well, the bulk degradation kinetics may cause some unpredicted mechanical failure of the construct.¹⁰⁹ Moreover, engineering scaffold microenvironments that highly mimic the cardiac ECM and fulfill all the requirements for a suitable engineered myocardium is still a big challenge.¹¹⁰

D. Early Perfusion Design in Tissue-Derived Acellular Scaffolds/Whole Hearts

Recently, acellular scaffolds derived from native tissues or organs have gained significant attention in the field of tissue engineering and regeneration.^{14,92,110–117} The purpose of decellularization is to remove all cellular components from the native tissue or organ, thus producing an ECM scaffold with its natural architecture, mechanical properties, and functional proteins.^{112,118–120} The removal of cellular content and xenogeneic and allogeneic antigens reduces foreign body reaction, inflammation, and potential immune rejection of the tissue-derived scaffolds.¹¹² The decellularization procedures, if chosen appropriately, can well preserve the complex 3D architecture, composition, and mechanics of the tissue-specific ECM, as well as numerous bioactive signaling factors.^{121,122} Many studies have confirmed that the tissue-derived scaffolds are biocompatible; optimal for cell

attachment, growth, and differentiation; and able to provide structural and biomechanical cues for constructive remodeling.^{112,118–120} Various decellularized ECMs, including small intestine submucosa (SIS), pericardium, skin, and heart valves, have received US Food and Drug Administration (FDA) approval and have been successfully used in both preclinical animal studies and human clinical applications.¹²³

In order to promote early perfusion, Chang et al.¹²⁴ created a bFGF-loaded porous acellular bovine pericardium; the scaffold was then recellularized with MSCs to repair the myocardial defect in a rat model. They found that the engineered construct improved the infarcted tissue regeneration, evidenced by vascularization and cardiomyocytes differentiation in the implanted region.¹²⁴ Wang et al.^{76,125} and Godier-Furnemont et al.¹²⁶ decellularized porcine (Figure 2A) and human myocardium and recellularized these acellular scaffolds with stem cells. Both studies found that the preservation of myocardial 3D ECM architecture and mechanical cues benefit cell proliferation, ingrowth, differentiation, tissue remodeling, and angiogenesis.^{76,126} To make a perfusable myocardial construct that fully utilizes the natural vascular tree in the myocardium, Schulte et al. made acellular myocardial flap scaffolds out of the porcine left anterior ventricular myocardium.¹²⁷ The decellularization was performed via the associated coronary artery and vein by perfusion method, which enabled the formation of a thick acellular myocardial flap scaffold with intact coronary arteries, veins, and microvasculature network (Figure 2B).¹²⁷

Besides the tissue level efforts in cardiac patches/constructs, whole heart decellularization and tissue engineering can be realized by coronary perfusion with detergents through the native vasculature to remove all the cellular components and leave a complete 3D ECM architecture with a full vascular network, which has the potential to establish blood supply to the possibly well-recellularized organ.^{112,128} For example, Ott et al.¹²⁸ successfully decellularized the whole rat heart, retaining the whole extracellular cardiac matrix with complete vascular tree, the intact atrial and ventricular geometry, as well as competent heart valves (Figure 2C).¹²⁹ After the acellular heart matrix was recellularized with cardiac and endothelial cells via a perfusion bioreactor, a revitalized beating rat heart, although with relatively weak pumping, was achieved.¹²⁹

One benefit of whole heart decellularization is that it has the potential to provide a complete perfusable vascular network and ventricular geometry for future recellularization and endothelialization. To optimize the recellularization of the heart vasculature system, Robertson et al.¹³⁰ recellularized the whole vasculature of an acellular rat heart with rat aortic endothelial cells (RAECs) using three strategies: retrograde aortic infusion, brachiocephalic artery (BA) infusion, or a combination of inferior vena cava (IVC) plus BA infusion. Their results showed that the combination (IVC + BA) cell reseeded method could enhance the vascular re-endothelialization when compared to the single-route strategies (Figure 2D).¹³⁰ Lu et al.¹³¹ have repopulated acellular mouse hearts with human iPSC-derived multipotential cardiovascular progenitor cells, and they reported good progenitor cell migration, proliferation, and differentiation in situ into cardiomyocytes, smooth muscle cells, and endothelial cells, of which the smooth muscle cells and endothelial cells are essential to successful revascularization. After 20 days of perfusion with iPSC-derived cardiovascular progenitor cells, the cell-repopulated heart tissues showed spontaneous

contraction and production of mechanical force, and they were also responsive to drugs.¹³¹ Current research also includes the scaling up to move the whole heart decellularization and revitalization from rat and mouse hearts to pig heart.¹³² For eventual application of the myocardium/whole heart decellularization approach, there still exist many technological challenges, such as large-scale expansion of cells, the thorough recellularization of a thick scaffold, the necessity to repopulate the entire tissue/organ, and full endothelialization of the complete vascular network.^{129,133}

E. ECM-Derived Hydrogel

Recently, injectable ECM hydrogels derived from decellularized tissues, such as myocardium and bladder, were found to be able to maintain the bioactivities of the tissue ECM, support cell repopulation, and promote constructive tissue remodeling.^{134–137} After tissue decellularization, the acellular ECM scaffolds are lyophilized, milled into fine powders, and digested with optimal concentrations of pepsin and hydrochloric acid (HCl) to make the pre-gel. Lastly, the ECM hydrogel forms, after adjusting the pH to 7.4 at 4°C and incubation at 37°C.¹³⁶ ECM hydrogels have a nanoscale architecture that is close to tissue ECM and have shown good biocompatibility for cell culture and stem cell differentiation, both as a thin substrate coating and as a thick gel.¹³⁸ Moreover, the injectable ECM hydrogel can be specifically derived from myocardium and hence preserve the appropriate biochemical compositions and mimic the myocardium specific ECM.^{134,135,137,139}

In the field of cardiac tissue engineering, the ECM hydrogel has been derived from the decellularized porcine ventricular myocardium, and this myocardial ECM hydrogel has been found to improve the maturation of ESC-derived cardiomyocytes in ECM hydrogel–cell co-culture.^{134,135} *In vivo* experiments with rats have further demonstrated that the myocardial ECM hydrogel can be successfully delivered via injection, and the gel formation/self-assembly was evident *in situ*.^{134,135} Furthermore, vascular cell migration and arteriole formation were found inside the myocardial matrix 11 days post-delivery of the myocardial ECM hydrogel into the rat left ventricular (LV) wall.^{134,135} Seif-Naraghi et al. further reported the safety and efficacy of the transendocardial catheter injection of the myocardial ECM hydrogel in a porcine model, demonstrating the potential of future clinical application.^{139,140} However, the ECM hydrogels do not provide strong mechanical support to the repaired region because of its gel nature.^{140,141} Moreover, this approach also faces the challenges such as the control of size, location, and fate of delivered materials, as well as the control of degradation profiles. The mechanical properties and degradation of the myocardium ECM hydrogel can be mediated through chemical cross-linking. Jeffords et al.¹⁴² used a natural cross-linker, genipin, to stabilize the ECM hydrogel, thereby increasing the mechanical strength and reducing the degradation rate. They found that 1 mM genipin cross-linked ECM hydrogel promoted vascular differentiation of hMSCs with significant expression of mature endothelial cell marker vWF without the need for exogenous growth factor addition. Their results suggest that improved angiogenesis could be induced by ECM hydrogel with tuned material properties.

V. BIOREACTORS FOR PROMOTING CELL DIFFERENTIATION, FUNCTIONAL PERFUSION, AND TISSUE MATURATION

Although some degree of success has been achieved in mimicking the morphological and functional features of native myocardium *in vitro*,¹⁴³ vascularization and angiogenesis still remain the two limiting factors in the application of tissue engineered cardiac constructs.^{64,144–146} Moreover, because of the relative thickness of the cardiac tissue constructs (more than a few millimeters) as compared to a cell sheet, transport of nutrients, oxygen, and other essential factors via diffusion is very limited.^{147,148} For example, the increased concentration of oxygen in the construct not only dictates the cell microenvironment but also improves the structural and functional properties of the construct.^{145,149} To overcome this mass transport issue during the development of the vascularized cardiac tissue constructs, a perfusion or multifaceted stimulation bioreactor is often utilized.^{150,151}

Angiogenesis in an engineered tissue constructs is a dynamic interplay of cell environment, cell-to-cell interactions, biochemical factors, and mechanical stimuli.^{152,153} By the use of perfusion bioreactor systems, mechanical forces can be introduced into the setup that simultaneously provides mechanical cues for initiation of angiogenesis and overcomes the mass transport issue.^{44,154–156} Perfusion bioreactors have been shown to produce a good cell seeding density, improved cellular environment, high cell viability, enhanced tissue microarchitecture, and increased oxygen distribution at the center of the cardiac tissue constructs.^{44,154–156} For cardiac tissue engineering endeavors, researchers often need not only a perfusion design, which provides the required nutrients or oxygen supply, but also the multifaceted stimulation, which provides appropriate mechanical and electrical stimuli to help stem cell differentiation, maturation of cardiomyocytes, and vascularization.^{154,157}

Carrier et al. applied the perfusion method to seed neonatal rat cardiomyocytes into PGA scaffolds in a rotating vessel microgravity bioreactor setup.¹⁵⁸ Using this method, the obtained cardiac tissue constructs achieved good cellular differentiation and ultrastructure.¹⁵⁸ Using a custom bioreactor based on the perfusion technique, Radisic et al.¹⁵⁹ seeded neonatal rat cardiomyocytes into a collagen scaffold (Ultrafoam) and achieved thick, compact, and viable cardiac tissue constructs. Hosseinkhani et al.¹⁵⁷ seeded rat cardiac stem cells into a composite collagen-PGA nanofibrous scaffold using a similar perfusion bioreactor, and the cellular proliferation achieved by this technique was better than those achieved by 3D static culture or 2D culture system. Furthermore, the type of perfusion system used in creating a cardiac tissue construct can also determine its contractile abilities.¹⁵⁹ Cardiac tissue constructs obtained from the pulsatile type of perfusion bioreactors (i.e., addition of pulsatile fluid motion) have produced better excitation thresholds and contractile properties than the cardiac constructs produced via the static or nonpulsated bioreactors.^{156,159} Similar pulsatile perfusion bioreactor systems for development of cardiac tissue constructs were also reported by Sodian et al.¹⁶⁰

The perfusion bioreactors incorporating electrical and mechanical stimulations are known as hybrid bioreactors.¹⁶¹ Barash et al.¹⁶¹ introduced an electrical stimulus to their perfusion bioreactor system for seeding neonatal rat cardiomyocyte onto an alginate scaffold.

Moreover, addition of the electrical stimulus to the culture was found to increase the striation of the cardiomyocytes, enhance the elongation of the cells, and increase expression of connexin-43 (cell-to-cell connection).^{145,147}

The perfusion bioreactor was also useful in the assembly of cardiac tissue constructs formed by multiple cell sheets, where an existing network of perfused blood vessels were used as the base for circulation of blood and nutrients, and the cardiac cells sheets were then layered one on top of another.¹⁴⁵ Similarly, Radisic et al.¹⁴⁷ used “capillary-like” channeled elastomeric scaffolds to provide oxygen and nutrients to their cardiac tissue constructs. These thin channeled scaffolds were first perfused with endothelial cells, and further stacked on top of one another to produce a thicker cardiac tissue construct¹⁴⁷; a similar study was also reported by Maidhof et al.¹⁶² In addition to perfusion of the medium and its constituents, Maidhof et al. further added mechanical and electrical stimuli to these channeled elastomeric scaffolds, and their resulting constructs had better cellular morphology, increased DNA content, enhanced cellular organization, and better cellular distribution.¹⁶³ Similar perfusion bioreactor systems, incorporating mechanical stretching and electrical stimulation, for cardiomyocyte culture was also reported by Kensah et al.¹⁶⁴ and Lu et al.¹⁶⁵ Other types of perfusion bioreactors for cardiac tissue engineering includes perfused microbioreactor arrays,¹⁶⁶ short term microperfusion bioreactors,¹⁶⁷ coupled transmural flow and axial flow perfusion systems,¹⁶⁸ bidirectional perfusion bioreactors,¹⁶⁹ and triple fusion perfusion bioreactors.^{128,170–173}

Recently, Wang et al.⁷⁸ applied multifaceted simulations on acellular myocardial scaffold-based cardiac construct, and found that the coordinated mechanical and electrical stimulations improved the efficiency of cell proliferation, differentiation, and maturation, as well as assisted constructive tissue remodeling that resulted in a biaxial mechanical behavior mimicking native myocardium. Morgan et al.^{174–177} further pointed out that the combined electrical and mechanical stimulations are necessary for improved functional properties plus the timing of the combined stimulation also greatly affect the engineered cardiac tissue. Moreover, a very interesting phenomenon was observed by Morgan et al.^{174,175}; they found that, when the electrical stimulus was slightly delayed after the beginning of mechanical stimulus, the yield constructs had improved function and better expression of proteins responsible for cell–cell communication and contractility. Hence, one subtle mimicking of the biophysical environment during isovolumic contraction is able to improve quality of the cardiac construct, implying that bioreactor design and the conditioning regimen planning should more accurately reproduce *in vivo* physiological conditions.^{174–177}

VI. URGENT NEED OF TISSUE ENGINEERING STANDARDS

From both engineering and business standpoints, the quality of a medical product cannot be compromised at accommodating any other expense in the entire product life-cycle. In order to deliver high quality products in a reliable way and reduce the additional cost associated with manufacture procedure, the standards of the quality control should be established for tissue engineering constructs. Moreover, the quality control standards should be strictly followed throughout the tissue engineering construct production procedure, from design, fabrication to evaluation, and from implantation to follow-up. The standardization of tissue

engineered medical products (TEMPs) ensures the repeatability, quality, and reliability of the final products for successful transition to clinical settings. Up to now, there are only several ASTM (American Society for Testing and Materials) and ISO (International Organization for Standardization) standards for preclinical evaluation of TEMPs (ASTM committee F4 is solely responsible for TEMPs). Approximately 20 other preclinical testing/evaluation standards have also been developed for TEMPs by either the Association for the Advancement of Medical Instrumentation (AAMI), the United States Pharmacopoeia (USP), or the American National Standards Institute (ANSI).¹⁷⁸ However, those standards have been formed by different institutes in response to a wide variety of needs, hence they lack overall systematic consideration. Moreover, few standards exist for TEMP production in the context of “scaling up” or in industrial manufacturing settings. Having a standardized method for each mentioned step (design, fabrication, evaluation, and implantation) can definitely increase the quality and reliabilities of the final products.

It is also notable that there is a large deficiency in standards consideration and implementation at the research/investigation level of TEMPs. Up to now, there exist only about 60 TEMP product patents that have followed ASTM standards. Another ironic fact is that the volume of papers published with consideration of ASTM standards is much less than the number of patents (ASTM standards are cited in approximately five tissue engineering papers versus ~2,000 TEMP patents).¹⁷⁹

Recently, more researchers appeal for the need of standards in tissue engineering endeavors. Nawroth et al.¹⁸⁰ summarized that, in order to scale up the TEMPs from design/production phase to a routine clinical setting, a systematic, reliable, and repeatable process that can be achieved via standardization is required. Standards for TEMPs should be the “common language” that all tissue engineering researchers should communicate with to work towards the common goal of manufacturing functionally and clinically relevant TEMPs in a scalable, controlled, and highly reproducible fashion. For example, for cardiac tissue engineering, standardization should be pursued in vital procedures such as cell isolation and culture, biomaterial synthesis, scaffold fabrication, cell-scaffold integration, cell/biomaterial injection, construct engrafting, early functional perfusion, and evaluation of regeneration and functional improvement. In simple words, development of standards and promotion of more cooperation among subject matter experts will increase reliability and productivity, reduce the complexity in TEMP production, reduce the commercialization burden, grow the TEMP industry, promote employment, and build revenue for future products.

VII. SUMMARY

The current approaches used in cardiac tissue engineering have their advantages and various degree of success in cardiac repair and regeneration, as well as in promoting angiogenesis and vascularization. However, those approaches in general have their limitations and challenges, such as difficulties in controlling the size, location, and fate of the implanted cells; limited thickness of the construct; challenges in identifying the ideal scaffold materials with proper biochemical and biophysical cues; insufficient vascularization before implantation; slow vascular network remodeling post surgery; poor integration with the host tissue; and the lack of efficiency in cardiac function improvement.

In the area of scaffolding and biomaterials, researchers have also experienced a variety of issues and hurdles. For instance, the nature-derived biomaterials often do not possess suitable mechanical properties, desired degradation profile, and tissue-specific architecture essential for cellular remodeling. Similarly, the myocardial ECM hydrogels face challenges such as the control of size, location, and fate of delivered materials, and the ECM hydrogels currently do not provide mechanical support to the repaired region. On the other hand, synthetic biodegradable polymers are facing challenges such as absence of biological signals, mismatched material properties, inflammatory response, and control of degradation profile. For whole heart tissue engineering, there are still technological hurdles that need to be addressed before successful application; those challenges include obtaining enough cells in large scale, thorough recellularization of thick ventricular walls, repopulating the entire heart with the right cell types, and full vascularization.

Among all the challenges, establishing functional perfusion and structure in the constructs/repairing regions is essential to the success of cardiac tissue engineering and regeneration. Without timely angiogenesis and effective vascularization, an engineered or repaired cardiac tissue can hardly sustain its functionality because of the lack of oxygen and nutrient supply, much less full integration with the host heart. Hence, the design of scaffolds, biomaterials, biofactor systems, or delivery procedures should not only consider the proper cells, cellular microenvironments, and biochemical and biophysical cues to drive the proper cell differentiations/tissue regeneration, but also the crucial need of angiogenesis and vascularization. Up to now, there is still a lack of highly accurate spatial and temporal control on both the recellularization and the angiogenesis/vascularization procedures in cardiac tissue engineering and regeneration.

To achieve more accurate spatial and temporal control, a combined strategy that harnesses the benefits of various approaches is more promising. For example, we recently proposed to reinforce cell sheets with acellular porcine myocardial scaffolds, with a goal to generate a more robust cardiac construct with good angiogenesis potential, which cannot be achieved by cell sheet technology alone. Moreover, in-depth knowledge on cardiac physiology, developmental biology, and even the cardiac pathophysiology should be considered when designing novel tissue engineering and regenerative strategies. As we discussed earlier, Morgan et al. further moved forward the concept of coordinated electromechanical simulations by more accurately mimicking isovolumic contraction (mirroring the physiology), which means slightly delaying the electrical stimulus at the beginning of the mechanical stimulus.^{174,175} More recently, 3D bioprinting has also been applied to print heart tissue (organoids) and blood vessels.¹⁸¹ One might envision that the future improvement attempts, either in establishing functional perfusion and structure or cardiac tissue development, should target mirroring the physiology closely¹⁸² and delivering building blocks with great temporal and spatial accuracy, which includes various types of cells, ECM/scaffolds, biofactors/molecules, microenvironment cues, perfusion, mechanical loading, and multifaceted biophysical stimulations.^{62,183,184} It is also worthwhile to point out that the immune response to implanted materials (e.g., nature-derived biomaterials, ECM/scaffolds, introduced cells, or combinations thereof) may play an important role in a proposed therapy's success.^{185–187} Moreover, the establishment and implementation of

tissue engineering standards is becoming an essential step to facilitate the translational applications of cardiac tissue engineering research from bench to bedside.^{62,183,184}

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FIG. 1. Strategies to establish early functional perfusion and structure in tissue engineered cardiac constructs.

FIG. 2.

(A) Well preserved, honeycomb-shaped cardiomyocyte lacunae and vascular channels (arrows) in acellular porcine myocardial scaffolds (H&E staining) (Wang et al., 2010). (B) Acellular porcine myocardial flap scaffolds with patent coronary arteries, veins, and microvasculature network (Schulte et al., 2013). (C) Vascular network of the decellularized rat heart (Otto et al., 2008). (D) Image of whole rat heart in which DiO-labeled endothelial cells delivered via the inferior vena cava (IVC) infusion and brachiocephalic artery (BA) infusion (Robertson et al., 2014). Figures reproduced with permission.

TABLE 1

Cell types used in cardiac tissue engineering and regeneration.

Cell Type	Advantages	Disadvantages
Skeletal myoblasts ¹	Can differentiate into cardiac-like myocytes Low risk of tumor formation	No synthesis of gap junctions
Cardiac stem cells ²	Can be self-renewed, expanded, and differentiate into both cardiomyocyte and vascular lineages	Cells isolated from aging hearts may not be capable to improve cardiac function sufficiently
Embryonic stem cells ³	Divide for unlimited passages in culture and can be differentiated into cardiomyocytes	Ethical conflict Possibility of teratoma formation Limited cell source
Induced pluripotent cells ^{4,5}	Have potential to differentiate into beating cardiomyocytes, smooth muscle cells, and endothelial cells	Potentially teratogenic Immature phenotype
Mesenchymal stem cells ³⁻⁵	Be able to regenerate myocardium, induction of angiogenesis, and free from ethical issues	Limited success in clinical