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Engineered heart tissues and induced pluripotent stem cells: macro- and microstructures for disease modeling, drug screening, and translational studies

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Abstract

Engineered heart tissue has emerged as a personalized platform for drug screening. With the advent of induced pluripotent stem cell (iPSC) technology, patient-specific stem cells can be developed and expanded into an indefinite source of cells. Subsequent developments in cardiovascular biology have led to efficient differentiation of cardiomyocytes, the force-producing cells of the heart. iPSC-derived cardiomyocytes (iPSC-CMs) have provided potentially limitless quantities of well-characterized, healthy, and disease-specific CMs, which in turn has enabled and driven the generation and scale-up of human physiological and disease-relevant engineered heart tissues. The combined technologies of engineered heart tissue and iPSC-CMs are being used to study diseases and to test drugs, and in the process, have advanced the field of cardiovascular tissue engineering into the field of precision medicine. In this review, we will discuss current developments in engineered heart tissue, including iPSC-CMs as a novel cell source. We examine new research directions that have improved the function of engineered heart tissue by using mechanical or electrical conditioning or the incorporation of non-cardiomyocyte stromal cells. Finally, we discuss how engineered heart tissue can evolve into a powerful tool for therapeutic drug testing.

Graphical Abstract

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Keywords

cardiovascular disease; disease modeling; drug screening; induced pluripotent stem cells; tissue engineering

1. Introduction

1.1 Cardiovascular disease

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in the United States [1]. Cardiomyopathies are a prevalent and serious class of CVD in which the contractile strength of the heart is compromised. Furthermore, although patients may present with similar symptoms, and hence are classified as suffering from a single disease, their underlying disease mechanisms are not uniform. Due to the variability in disease mechanisms, medications and dosages may vary among patients, making it difficult for caregivers to provide universally efficacious treatment, thus creating a tremendous burden on the healthcare system. Furthermore, the development of a new drug is expensive and inefficient, requiring on average over a decade of research and development and ~\$5 billion per drug [2-6].

1.2 Drugs withdrawn from the market due to cardiac toxicity

Withdrawal of drugs from the market due to previously unobserved toxic effects in animals (or false negatives), which have been attributed to off- and on-target toxicity including cardiotoxicity, is an unfortunate reality [7-10]. As examples, Terfenadine (trade name Seldane) was withdrawn in 1998 for inducing cardiac arrhythmias; Grepafloxacin (trade name Raxar) was withdrawn in 1999 for prolonging the QT interval; Rofecoxib (trade name Vioxx, Ceoxx, and Ceeoxx) was withdrawn in 2004 for the risk of myocardial infarction; and Rosiglitazone (trade name Avandia) suffered from a drastic decrease of sales due to reports of increased risk of heart attack [3, 9]. Typically, drug safety and efficacy are evaluated using non-human animal models followed by costly clinical trials. Human models are difficult to establish because human heart cells or tissue from patients cannot survive long-term culture [11] and are difficult to obtain [12, 13]. The existing preclinical testing paradigm relies heavily on the use of *in vitro* cell lines such as Chinese hamster ovary

(CHO) and human embryonic kidney 293 (HEK293) cells overexpressing the human ether-à-go-go-related gene (hERG) channels, *ex vivo* tissue preparations such as isolated arterially perfused left ventricular rabbit wedge preparations, and *in vivo* studies such as chronic dog atrioventricular (AV) block models [10]. However, there are several challenges with these models, including their high costs and their poor predictive capacity owing to inter-species differences in cardiac electrophysiology and human biology [14, 15]. In addition, CHO and HEK293 cells are not ideal models for cardiotoxicity because ectopic expression of a cardiac ion channel does not always recapitulate function in human cardiomyocytes [16]. Models with poor predictive power lead to a high probability of discarding new chemical entities (due to false positives) that otherwise might have become safe and effective drugs. Hence, there is a need for immediate attention from all stakeholders involved in the drug discovery process to address these concerns and to better evaluate drugs before clinical trials.

1.3 Induced pluripotent stem cells for disease models

A new approach towards reducing inefficacious drug treatment is precision medicine, and this endeavor is increasingly feasible with the advent of induced pluripotent stem cells (iPSCs) [17, 18]. Unlike other cells, iPSCs reflect a person's unique genotype because they are derived from a patient's somatic cells (e.g., peripheral blood mononuclear cells or skin fibroblasts). They have the capacity to differentiate into all cell types, including cardiomyocytes (CMs), the force-producing cells of the heart [19, 20]. Patient- and disease-specific models are being developed to provide unprecedented multi-dimensional information on the individual's disease and a system to evaluate innovative therapeutic options.

Patients carrying known mutations for a disease are able to contribute to the generation of disease-specific iPSC lines. For example, some of the first iPSC-derived cellular models were developed for LEOPARD syndrome [21], long QT [22, 23], familial dilated cardiomyopathy (DCM) [24], familial hypertrophic cardiomyopathy (HCM) [25], Timothy syndrome [26], and aldehyde dehydrogenase 2 genetic polymorphism [27]. Channelopathies, caused by specific mutations in cardiac ion channels, can also be modeled using iPSC-CMs. One example is long QT syndrome, which is characterized by prolonged ventricular repolarization that can lead to sudden cardiac death [28, 29] and is caused by mutations in potassium channels [30].

The quality of the disease model can be determined by the disease phenotype of the iPSC-CM as compared to the physiological disease phenotype. For example, DCM iPSC-CMs carrying the TNNT2 mutation [31] displayed disorganized sarcomeric structures, abnormal calcium handling, and decreased contractile function similar to the cardiomyocyte phenotype in DCM patients. Likewise, iPSC-CMs from patients with an HCM mutation in the myofilament myosin heavy chain 7 (MYH7) [25] recapitulated phenotypic features of abnormal calcium handling, increased myofibril content, and cellular hypertrophy at baseline and upon stress [25].

A disease model must also recapitulate physiological drug response in order to accurately evaluate drugs before treatment administration. For example, iPSC-CMs derived from DCM patients respond to β agonists and β blockers in a similar manner to patients. It is important

to understand patient-specific β blocker response because although β_1 -specific adrenergic blockers are beneficial to patients with DCM [32, 33], the use of β -adrenergic agonists can lead to increased morbidity and mortality in patients with heart failure [34]. Upon exacerbation by β -adrenergic agonists such as norepinephrine (a drug which physiologically activates the fight-or-flight response and increases heart rate), the DCM iPSC-CM model recapitulates the DCM phenotype [31]. After adding the β -blocker metoprolol, the phenotype was rescued, recapitulating results from previous β -blocker trials [32, 33]. In a recent mechanistic study using the DCM iPSC-CM model, a mechanism of compromised β -adrenergic signaling was identified as nuclear localization of mutant TNNT2 and epigenetic regulation of phosphodiesterases 2A and 3A [35].

In another example, iPSC-CMs with long QT mutations had a similar drug response as patients with long QT. Arrhythmias were induced upon treatment with β -adrenergic stimulants and arrhythmias were rescued upon subsequent addition of β blockers [22, 36]. In a drug response study, an iPSC-CM model derived from long QT patients showed shortened repolarization upon treatment with the experimental potassium channel enhancers nicorandil and PD118057 [36]. Based on these results, the use of potassium channel enhancers might be considered in patients with long QT.

1.4 Human iPSC-CMs as compared to neonatal and adult cardiomyocytes

The quality of human iPSC-derived cardiomyocytes (iPSC-CMs) is determined by the maturity of their structure and function as compared to human neonatal or adult cardiomyocytes (**Fig 1**). (The same criteria also apply to human embryonic stem cell-derived cardiomyocytes, or ESC-CMs). It is well known that independent of cell line or reprogramming and cardiac differentiation methods, the resultant iPSC-CMs possess gene expression, structural, and functional properties that resemble the human fetal CM phenotype more than adult CMs [37-41]. For example, iPSC-CMs display expression of genes implicated in the mechanical and electrophysiological manifestation of cardiac contraction, including *MYL2*, *MYH7*, *TNNI3*, *SCN5A*, and *KCNH2*. The expression of sarcomeric protein *MYH6* approaches adult levels, whereas the expressions of *MYL7*, *MYH7*, and *TNNI3* are relatively less in iPSC-CMs compared to both fetal and adult cardiomyocytes [38, 42-44]. Structurally, iPSC-CMs are smaller in size and have irregular shapes (round/oval rather than rod-like shapes) compared to adult human cardiomyocytes [40, 45]. In addition, iPSC-CMs show unorganized sarcomeres, and the absence of T-tubules results in much slower excitation-contraction coupling [24, 39, 46].

The functional similarities and differences of iPSC-CMs to adult CMs also exist in the electrophysiological properties of the cells. These differences are used in assessing the maturation of the iPSC-CM (**Fig 1**). For instance, freshly dissociated human adult CMs are quiescent and beat or contract in response to stimuli [39], while ventricular- and atrial-like iPSC-CMs are autonomous and spontaneously beat which is due to depolarized resting membrane potential. This increased automaticity of iPSC-CMs can be attributed to the presence of large funny currents (I_f) or pacemaker currents in addition to the under-expression of the inward rectifier potassium channel (I_{K1}) consequentially leading to a large inward sodium current [39, 47]. Moreover, present cardiac differentiation methods produce

a mixed population of ventricular-, atrial-, and nodal-like cells with ventricular-like CMs being the predominant cell type [16, 25, 48-54]. These three highly distinct subpopulations possess a spectrum of action potentials with varying features: maximum diastolic potential (MDP, -57 to -75 mV); action potential amplitude, (APA, 87 to 113 mV); action potential duration at 90% repolarization (APD₉₀, 173.5 ± 12.2 to 495 ± 36 ms); maximal upstroke velocity (dV/dt_{max}) (9 to 40 V/s); mean duration potential [22, 26, 54-56]. This heterogeneity corresponds to various stages of development and maturity as corroborated by single cell transcriptional profiling and is also suggestive of significant inter-cell line variability [39, 57]. However, this heterogeneity also represents a potential limitation in modeling cardiac diseases where a specific cardiomyocyte type is affected or in high-throughput drug screening.

Several studies have devised strategies to increase the maturity of iPSC-CMs, such as extended time in culture, overexpressing a deficient ion channel (e.g., I_{K1}) [58] or cardiac specific microRNA (e.g., miR-1/miR-499 [59], let-7 [60]), pharmacological and neurohormonal treatment (e.g., adrenergic stimulation and triiodothyronine) [39], or electrical/mechanical conditioning (**Fig 1**). These approaches promise to significantly increase the overall maturity of iPSC-CMs. Further discussion of maturation strategies of iPSC-CMs using biochemical and molecular biology approaches is beyond the scope of this review, and interested readers are encouraged to refer to other reviews [29, 39, 41, 61].

1.5 iPSC-CMs for patient-specific 3D engineered heart tissue models

The ability to fabricate engineered heart tissue from diseased or healthy iPSC-CMs has led to its potential use as a tool in disease modeling, disease diagnosis with genotype-phenotype biomarkers, efficacy testing, cardiotoxicity and safety pharmacology, and the identification of disease-associated genes (**Fig 2**). The information learned from engineered heart tissues is foundational for future endeavors to create patient-specific heart tissue replicas for drug screening prior to treatment. Human iPSC-CMs can be utilized to fabricate 3D model structures that serve as models of cardiac tissue [62]. 3D models require a high number of cardiomyocytes, which is now more accessible with recent advances in robust and efficient cardiomyocyte differentiation protocols [17, 19, 20, 63]. With the increased availability of raw materials, the current challenge is to achieve physiologically relevant formation of engineered heart tissue.

2. Macrostructures of engineered heart tissue

2.1 Cardiomyocyte aggregates: the first engineered heart tissue

Primary cardiomyocytes cultured in aggregate demonstrate improved function compared to single cells [64]. By contrast, floating aggregates of cardiomyocytes are functionally more similar to intact heart tissue than 2D-monolayer cultures [65]. Collagen has become a standard hydrogel for 3D cardiomyocyte culture [64]. Building on work originally performed to prevent the detachment of skeletal myoblasts in culture [66], cardiomyocytes have been embedded in collagen I. The 3D environment of collagen improved tissue formation, inhibited dedifferentiation and overgrowth by non-cardiomyocytes, and allowed for the detection of contractile forces from multi-cellular samples [64, 67].

Following these studies, the format of engineered heart tissue has been evolving with the primary objective of improving electromechanical function (**Table 1**). The creativity displayed in the various designs of engineered heart tissue has led to increased functional readouts and a deeper understanding of engineered heart tissue formation and function.

2.2 Stretch-induced contractility of engineered heart tissue

There is a critical relationship between cardiac tissue structure and contractile function. CMs interact via gap junctions, which facilitate the conduction of electrical signals [68, 69] and contractile forces [70]. Cell-cell interactions are important for the distribution of internal forces such as stretch, which occurs in the heart as a result of ventricular filling. Consequently, contractile forces are enhanced by passive forces experienced by the heart while under stretch [71-74]. This length-dependence of force generation, known as the Frank-Starling relationship [75-78], is evident in engineered heart tissue from native rat cardiomyocytes [79, 80] as well as stem cell-derived engineered heart tissue [73]. Total force per cross-sectional area generated by the tissue (stress) can be measured while the tissue is gradually stretched in fixed intervals (strain) [64]. This stress versus strain analysis yields a biomaterial property called the Young's modulus, which describes the stiffness of the tissue [81].

Macrostructures of engineered heart tissue that are matured under passive stretch increase strength of contraction (**Table 2**). One of the earliest macrostructures of engineered heart tissue that was developed under passive stretch was fabricated by Eschenhagen et al. using chick embryonic ventricular myocytes in a collagen hydrogel [64, 79]. The force of contraction of paced engineered heart tissue remained remarkably stable for up to 20 hours, unlike intact muscle preparations. In subsequent experiments, engineered heart muscle (EHM) was fabricated from native rat cardiomyocytes in collagen, resulting in 1 mm thick constructs [67, 74, 79, 82]. Their ring shape facilitated positioning onto two-pronged stretchers to allow for constant passive stress. They were visibly beating and produced 0.3 mN of force [80]. In a different report, the maximum static tension and total force were found to be similar at 0.4 mN and 0.48 mN [73]. For comparison, *ex vivo* rat heart tissue exerts 48.8 ± 5 mN in contractile force [83]. Cardiomyocyte alignment is also observed in stretch-induced engineered heart tissue [67, 73, 84, 85].

Passive stretch conditions have also improved the function of iPSC-derived and ESC-derived engineered heart tissue developed as collagen-based hydrogels [73]. Upon uniaxial cyclic stress is reminiscent of the cyclic, pulsatile stretch of the beating heart, cardiomyocyte size and proliferation increased, and force generation was length dependent [73].

2.3 Mechanical and electrical conditioning of engineered heart tissue

Maturation of the tissue can be advanced with mechanical and electrical conditioning (**Table 2**). In the heart, contractile forces $F_{\text{contraction}}$ are enhanced by stretch (i.e., the stretch of the ventricle upon filling) [86] [72, 73]. Electromechanical coupling is evident in collagen-based engineered tissues, which beat spontaneously and synchronously when exposed to static stress [61] [73]. This force generation is evident in constructs formed from either rat neonatal ventricular cardiomyocytes or pluripotent stem cell-derived cardiomyocytes (PSC-

CMs which include both ESC-CMs and iPSC-CMs. For example, in one report, engineered heart tissue produced a passive stress of 0.36 ± 0.06 mN and a total force of 0.75 ± 0.11 mN [80]. In comparison, left ventricular heart muscle strips derived from the adult human heart have been reported to exert 21.7 ± 2.6 mN/mm² of stress at maximum length [87]. Other examples of force generation profiles in engineered heart tissue are detailed in Table 2. Overall, mechanically conditioned engineered heart tissues can produce approximately up to 2-5% of the force compared to native tissue.

Electrical stimulation also conditions engineered heart tissue. Exogenous electric fields have been implicated in cardiac differentiation [88]. For example, electrical stimulation of collagen-based engineered heart tissue has resulted in ultrastructural organization and increased contractile force [85]. In another report, biphasic electrical stimulation of microtissue improved organization, elongation, and the presence of gap junction protein Connexin-43 [89]. Electrical field stimulation of biowires improved ultrastructural organization, conduction velocity, calcium handling, and electrophysiological properties [90]. Recently, optogenetics has been developed as a tool to induce action potentials using light stimulation [91]. Control of light-gated sodium channel with channelrhodopsin-2 induces a transmembrane potential leading to the propagation of an action potential [92]. This optogenetic tool initiates the process of excitation-contraction coupling, thereby acting as an “on-switch” for contraction [93]. Halorhodopsin is a light-gated pump for chloride ions that acts to repolarize and return ions to resting membrane potential [94]. Therefore, these genetic tools potentially can be used to mature cardiomyocyte contractile function through mechanical conditioning with light-controlled contraction.

2.4 Electrical function of engineered heart tissue

The quality of iPSC-derived heart tissue models is determined by its structure and function as compared to native human adult heart tissue. Quantitative measures of the functional maturity of engineered heart tissue include action potential properties, such as resting membrane potential, minimum excitation threshold, and conduction velocity. The overall electrophysiology of engineered heart tissue is less mature than native human heart tissue.

The resting membrane potential is an inherent property of the cell [95]. The mean ventricular and atrial resting membrane potentials of native human heart have been respectively reported at -87 mV and -70 mV; action potentials exist with both a prominent spike and a plateau [96]. For engineered heart tissue, action potential recordings are recorded on a representative cell population at the time of seeding [50] or on isolated cardiomyocytes after tissue formation [90, 97]. The resting membrane potential of CMs from engineered heart tissue ($E_k = -96$ mV) is hyperpolarized compared to that of the monolayer CM (approximately -60 mV) [98]. Based on initial work done with rats, action potential recordings from engineered rat heart tissue displayed stable resting membrane potentials of -66 to -78 mV, fast upstroke kinetics, and a prominent plateau phase [80]. Sharp electrode electrophysiology would be necessary to record resting membrane potentials from intact engineered heart tissue.

Engineered heart tissue has been reported to generate both spontaneous and elicited action potentials, and the minimum voltage (via electrical field stimulation) to elicit synchronous

beating is referred to as the minimum excitation threshold (MET) [99]. MET is higher in engineered heart tissue (2.97 ± 0.30 V) compared to adult ventricular heart tissue (1.34 ± 0.17 V) [99]. MET can be reduced closer to physiological levels after electrical conditioning and consequent improvement of excitation-coupling [89]. However, others have demonstrated that the MET remained unaffected by electrical pacing [100]. Cellular composition affects the MET, such that constructs with 100% CMs had a MET of 3.2 V/cm whereas constructs with 60% CM purity had 5 V/cm [101]. Furthermore, MET is also affected by tissue orientation and tissue heterogeneity, which may introduce secondary excitation sources and incorrect low excitation thresholds [102, 103]. Thus, heterogeneities in the engineered heart tissue as well as electrode and bath geometry are important considerations when interpreting the electrical function of engineered heart tissue.

In conjunction with MET, the extent of gap junction coupling affects the kinetics of repolarizing and depolarizing currents and is reflected in the maximum capture rate (MCR), or the maximum rate at which engineered cardiac tissues can be steadily stimulated by a point electrode [104]. In response to electrical field stimulation, the MCR is approximately 180 to 270 beats/min [101]. In comparison, the MCR for neonatal and adult ventricular tissue is greater at 475.0 ± 25.0 and 281.2 ± 21.0 beats/min, respectively [99]. Native tissue has a higher MCR and a lower MET compared to engineered tissue.

The conduction velocity of the propagating action potential is a metric of cardiomyocyte gap junction coupling as well as the initiation of the action potential, which is dependent on both the sodium conductance and the resting membrane potential [104]. The conduction velocity is approximately one-third lower in engineered heart tissue compared to native adult human ventricular tissue: native adult human ventricular tissue has a conduction velocity of 45 cm/s [105], or in a more recent report, 31.69 ± 4.44 cm/s [99], whereas the recorded conduction velocity of engineered heart tissue is 11.89 ± 0.46 cm/s [50, 99]. However, the conduction velocity is dependent on CM composition and can be increased to approximately 25 cm/s at 90% hESC-CM purity [50]. Interestingly, conduction velocities are greater in 3D engineered heart tissues compared to age- and purity-matched 2D monolayers composed of 48-65% CM (9.76 ± 1.0 cm/s vs 5.17 ± 0.7 cm/s) [50]. In addition to cardiomyocyte purity, conduction velocities increase upon electrical stimulation [90].

Overall, the electrophysiological function of 3D-engineered heart tissue resembles physiological properties more closely than 2D counterpart models, as evident by various electrophysiological parameters such as resting membrane potential, minimum excitation threshold, and conduction velocity.

3. Preparing engineered heart tissue for transplantation

3.1 Prevascularization of engineered heart tissues: on route for scale-up

During the development of heart tissue, the coronary circulation provides important factors through perfusion and paracrine signaling. There is strong motivation to pre-vascularize the *in vitro* generated tissues in order to achieve macrostructures of engineered tissue constructs with longer *in vivo* graft survival [106]. One approach has been to incorporate endothelial cells. Tulloch et al. have reported the formation of lumen-containing structures when adding

human endothelial cells to human ESC-CMs and fibroblasts [73]. Determining the appropriate mixture of cells will be critical in developing engineered heart tissues that most closely mimic native heart tissue. There are no reports to date of vascular function or increased function of the cardiac tissue construct due to pre-vascularization with endothelial cells. However, there has been progress with the development of a vascular patch, consisting of human ESC-derived endothelial cells and smooth muscle cells in fibrin, which has been shown to significantly improve LV function in a porcine model of post myocardial infarction [107].

3.2 Translational studies for integration and function

Animal models of transplanted engineered heart tissue have provided valuable insight regarding graft integration and *in vivo* function. Initial studies focused on implanted engineered heart tissue developed from neonatal rat cells ($8 \times 10 \times 1$ mm) around the circumference of hearts from syngeneic and immunosuppressed rats [74, 79]. Functionally, implanted EHMs did not improve left ventricular function; however, they were heavily vascularized after 14 days [74, 79]. When engineered heart tissue containing human cells (cardiomyocytes, endothelial cells, and stromal cells) were transplanted into athymic rats, the graft survived and formed human microvessels perfused by the host coronary circulation. Certainly, vascularization will be crucial for long-term survival and integration of the graft into the host [108].

Engineered heart tissue has also been implanted into injured heart models. Engineered heart tissue developed from neonatal heart cells (thickness/diameter, 1-4 mm/15 mm) was transplanted into immunosuppressed infarcted rat hearts [84]. The engineered heart tissue survived after implantation and supported contractile function of infarcted hearts, including electrical coupling to the native myocardium as well as physiological improvements such as the prevention of further dilation, induced systolic wall thickening of the infarcted myocardial segments, and improved fractional area shortening [84].

A porcine model of acute myocardial infarction has also been reported with the transplantation of iPSC-derived cardiac, endothelial, and smooth muscle cells that were embedded in a fibrin patch and insulin growth factor (IGF)-encapsulated microspheres [109]. Results showed integration of the engineered heart tissue into the host myocardium, generation of organized sarcomeric structures, and improved physiology (i.e., left ventricular function, myocardial metabolism, and ventricular wall stress, amongst others). This porcine study highlights the potential of utilizing human iPSC-derived cells for cardiac repair. It has also been shown that human ESC-CMs can be transplanted and can survive in non-human primate models of myocardial ischemia followed by reperfusion [110]. Animals with grafts demonstrated perfusion of the graft by the host vasculature, regular calcium transients, electromechanical synchronization to the host myocardium, and remuscularization of the infarcted heart [110]. This study demonstrated important phenomena of survival and integration of the cells into the host, raising the possibility that engineered heart tissue derived from these cells could further improve cell retention and functional improvement of the infarcted heart. Noninvasive imaging techniques that can

follow the survival of these engrafted cells would be helpful in optimizing these preclinical studies [111, 112].

Results of iPSC-CMs have led to the initiation of the first human clinical trial using ESC-derived cardiac progenitors in a fibrin gel for patients with ischemic heart failure (ESCORT, NCT02057900). ESC-derived cardiac progenitors are sorted for CD15⁺/Isl1⁺ [113] and delivered at the epicardium. The trial is currently recruiting participants [114] and is designed to provide critical insight into the safety of engineered heart tissue in patients with heart ischemia.

4. Microstructures of engineered heart tissue

4.1 Microstructures to improve oxygen and nutrient diffusion rate limitations

The size of engineered heart tissue will be an important consideration for future clinical needs as a graft for the human heart. However, the size of engineered cardiac tissue is limited by the rate at which diffusion can deliver oxygen and nutrients to its core because cardiomyocytes cannot tolerate hypoxia for prolonged periods of time. Diffusion rates are sufficient up to a thickness of 100-128 μm [115, 116]. One solution is to rely upon vascularization of the graft by the host after implantation. However, this approach requires time and is suboptimal for the survival of the graft. Another approach is to decrease the size of the graft to dimensions that enable nutrient exchange solely by diffusion. The optimization of oxygen and nutrient transfer will improve long-term graft survival and host integration.

Microtissues engineered into microfluidic platforms can provide continuous nutrient exchange. The controlled organization of cells and their microenvironments have led to the development of engineered vascular perfusion systems (Table 1). Microphysiological systems (MPS) have been reported as 2D models for cardiac tissue that allow for gas and nutrient exchange via microfluidic channels surrounding the microtissue [3]. In MPS, cardiomyocytes are aligned, thus providing physiologically relevant microtissue structures. Because the continuous flow in the microfluidic chambers allows for drug testing, MPS is an exemplary model for predicting drug-induced toxicity [3].

Perfused bioreactor systems can also enhance mass transport between culture medium and cells of engineered cardiac constructs. Different vessels, including rotating vessels and mixed flasks for laminar and turbulent flows [117] as well as perfusion reactors [118], have been utilized successfully. Cardiac cell-gel constructs that were generated from neonatal mouse cardiomyocytes and collagen and that were cultured in perfusion reactors maintained a constant frequency of contractions upon electrical stimulation, whereas constructs in rotating vessels exhibited episodes of arrhythmia [118]. It is not surprising that fluid mechanics plays a critical role in the development and function of heart tissue. After all, the heart is a highly dynamic organ with complex flow profiles. Given that bioreactor systems are nascent technologies for cardiac tissue engineering, continued research will be critical in understanding the effects of fluid flow on tissue, cellular, and molecular levels for incorporation into engineered heart tissue models.

4.2 Microtissues for force generation

Microtissues capture the phenomena of interactions between cells and the environment, and therefore are used to assess important cardiac properties *in vitro*. Methods to measure functional output (i.e., contractile force and action potential generation) are integrated into these systems. Heart-on-chip technology is a prime example of functional microtissues [119]. In this high throughput system, each sample consists of a thin sheet of cardiomyocytes. As the sheet contracts during contraction, deflections in tissue position are measured using image detection techniques, and subsequently, the amount of force generated can be calculated. Within the same system, action potential propagation can be measured. Heart-on-chip technology, thereby allowing high throughput analysis of mechanical and electrical output of a 2D cardiac tissue model. Similar studies have been done with microtissues generated from rat neonatal ventricular myocytes and collagen and around flexible posts; forces were calculated from beam bending theory based on the stiffness of the posts and ranged from 6-15 μN [100]. Overall, microtissues provide valuable models for studying a three-dimensional phenomenon of force generation without the limitation of diffusion-limited mass transfer.

5. iPSC-derived 3D models for disease modeling and drug screening

The use of iPSC-derived cells for tissue engineering is a nascent field. There is great promise in these tissues because they reflect the 3D interactions between cells and the environment within a personal genotype. Most recently, microtissues have been developed to model DCM [120]. Findings using the 3D model indicate that certain titin mutations are pathogenic and cause DCM by disrupting critical linkages in the sarcomere, diminishing contractile properties, and impairing response to mechanical and β -adrenergic stress [120]. In another example, a microstructured 3D model of iPSC-CMs has been used to model Barth syndrome, a mitochondrial myopathy of the myocardium and skeletal muscle caused by a mutation in the gene Tafazzin (TAZ) [121]. iPSC-CMs carrying the TAZ mutation were studied using heart-on-chip technology and were noted to have structural sarcomeric abnormalities and weakened contraction; they also displayed lower contractile stresses, recapitulating the Barth phenotype in patients. Phenotypic rescue was achieved using small molecule treatments [121].

Engineered heart tissue is also being developed for drug screening, in particular microphysiological systems (MPS) using human iPSC-CMs. Pharmacological agents have been tested in these MPS, including isoproterenol (β -adrenergic agonist), E-4031 (hERG blocker), as well as two drugs with known clinical effects, verapamil (multi-ion channel blocker) and metoprolol (β -adrenergic antagonist) [122]; beat rate and mechanical motion were used as metrics of cardiac tissue function.

6. Limitations of current engineered heart tissues

The predictive abilities of engineered tissue systems are still limited, as these constructs do not recapitulate the human *in vivo* structure and organization of the myocardium. The structure of engineered heart tissue more closely resembles the organization of neonatal heart tissue. Native heart beat rates can be achieved, but maximum force produced per cross-

sectional area of tissue is only 1% of native tissue. The stiffness, as indicated by the Young's modulus, of engineered heart tissue is half of native tissue, which is about 200 kPa [123]. Although microtissues circumvent the issues of diffusion-limited mass transfer, size of engineered tissue will become important in the field of precision medicine. Accordingly, it will also be important to understand whether the tissue construct will couple to the native heart. Encouragingly, in rat studies, electrical pacing of an implanted engineered heart tissue resulted in remote electrical propagation in the myocardium, suggesting coupling of the engineered heart tissue graft to the host myocardium [84]. As the field of tissue engineering continues to grow, established metrics will facilitate the comparison between designs. The ability to compare the quality of iPSC-CMs across laboratories will be possible with an established set of standards [45]. For example, in order to compare force measurements across models and laboratories, a common method for reporting force (i.e., stress/cell) will need to be adopted. This model will need to capture information about the force distribution throughout the tissue as well as the load experienced by each cell. These standards should address genetic, structural, electrophysiological, and contractile parameters as compared to a native ventricular cardiomyocyte [124].

7. Conclusions

There has been great progress in developing a heart tissue model that can provide force output – the ultimate functional readout of the myocardium. Microsystems provide mechanical and/or electrical conditioning in a micro-sized environment that enables maturation of the engineered tissue while incorporating cell and environmental interactions and functional readouts of force and/or electrophysiology. Scaling the tissue construct has been possible with technological advances in iPSC generation and iPSC-CM differentiation. Diffusion-limited nutrient delivery is addressed technologically with incorporated systems of controlled rate mass exchange or biologically with the inclusion of cells for tissue pre-vascularization.

The future direction of engineered heart tissue technology will be with combined research efforts in the areas of patient-derived cells and engineered tissues. This platform could be used to assess novel drug effects and screen for deleterious effects such as arrhythmias, impaired contractile force, and cardiotoxicity; therefore, it could serve as a valuable tool for new drugs before clinical trials.

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Fig 1. Maturation and assessment of stem cell-derived cardiomyocytes

Many strategies have been reported for the maturation of cardiomyocytes. In the biochemical approach, growth hormones or adrenergic agonists are added to promote a change in cardiomyocyte function. With molecular biology approaches, cardiac specific ion channels and microRNA are overexpressed to elicit changes in the electrophysiology and calcium handling of cardiomyocytes. Bioengineering approaches have also been shown to improve sarcomeric organization and contractile function by incorporating controlled parameters of substrate stiffness, topography, electrical/mechanical conditioning, and integrated systems that improve nutrient delivery. Assessment of function ranges from examining morphology (i.e., cell shape/size, sarcomeres, T-tubules, and alignment), to molecular assays (sarcomeric and ion channel expression), and functional assays (i.e., calcium transients, electromechanical coupling, contraction, electrophysiology, and tissue grafting).

Fig 2. Application of engineered heart tissue model

Engineered heart tissue can be fabricated from diseased or healthy patient-specific iPSC-CMs. They are being utilized as models for various applications including modeling disease pathogenesis, disease diagnosis with genotype-phenotype biomarkers, pharmacological screening/efficacy testing, cardiotoxicity/safety pharmacology, and the identification of disease-associated genes.

Fig 3. Current protocols in engineered heart tissue

The most current engineered heart tissues include cardiomyocytes and an organic/synthetic scaffold, and sometimes also endothelial cells and supporting cells. The advent of iPSC-CMs makes it possible to fabricate patient-specific engineered heart tissues. There are various forms of conditioning, including mechanical stress (static and cyclic) and electrical stimulation. Measurable outputs include structural, mechanical, and electrical information as well as *in vivo* graft performance (survival, integration, and electromechanical coupling with the host).

Table 1

Various formats of engineered heart tissue and their functional readouts

Shape	Size	Cells	Scaffold	Supporting cells	Conditioning	Functional readout	Ref
Band	1.5 × 1.5 × 12 mm	NA	Native human left ventricle	None	None	Contractile force	Mulieri et al., 1992
Band	15 × 17 × 4 mm	Embryonic chick V-CM	Collagen	None	None	CM structure Contractile force	Eschenhagen et al., 1997
Ring	D/T 8-10 mm / 1 mm	Neonatal rat V-CM	Collagen	None	Passive stress	Contractile force <i>In vivo</i> engraftment	Zimmermann et al., 2002
Bioreactor	n/a	Neonatal rat V-CM	None	None	Turbulent flow	Metabolic activity Ultrastructure	Carrier et al., 2002
Bioreactor	D/T 1.1 mm / 1.5 mm	Neonatal rat V-CM	Collagen	None	Laminar flow	Cell viability	Radisic et al., 2003
Band	6 × 8 × 1.5 mm	Neonatal rat V-CM	Collagen	None	Electrical	CM structure Contractile properties	Radisic et al., 2004
Ring	D/T 15 mm/ 1-4 mm	Neonatal rat V-CM	Collagen	None	Passive stress	<i>In vivo</i> grafting on infarcted hearts Electrical coupling	Zimmermann et al., 2006
Micro tissue	D/T: 100-200 µm / 3-4 mm	Neonatal rat V-CM	Collagen	Fibroblasts ECs	Electrical	CM structure Electrophysiology	Chiu et al., 2011
Band	20 mm × 0.5 mm	hESC-CM hiPSC-CM	Collagen	Stromal (MEFs or HUVEC-ECs)	Mechanical (Static stress)	Contractile force <i>In vivo</i> graft survival Host vascularization	Tulloch et al., 2011
Micro tissue	D/L 100 µm / 500 µm	Neonatal rat V-CM	Collagen	None	Electrical	Contractile force Calcium dynamics	Boudou et al., 2012
Patch	7 × 7 × 1.5 mm ²	hESC-CM	Fibrin	45-90% CM purity	Unidirectional passive stress	Gene expression Electrophysiology Contractile force	Zhang et al., 2013
Biowire	600 µm diameter	hESC-CM hiPSC-CM	Collagen	None	Electrical	Structure Electrophysiology Calcium dynamics	Nunes et al., 2014
Patch	n/a	hiPSC-CM	Fibrin	ECs, SMCs	None	<i>In vivo</i> survival / integration	Ye et al., 2014
Thin film	95 µm × 13 µm	hiPSC-CM	None	None	None	CM Metabolism CM Structure Contractile force	Wang et al., 2014
MPS	100-200 µm	hiPSC-CM	None	None	None	Drug response	Mathur et al., 2015

CM, cardiomyocytes; D, diameter, EC, endothelial cells; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; MPS, microphysiological systems; NA, not applicable; SMC, smooth muscle cells; T, thickness, V, ventricular-type.

Table 2

Contractile assessments of engineered heart tissue

Cells	Cell Number	Size	Force Output			Beat Rate	Ref
			Passive Force	Contractile Force	Total Force		
Native rat ventricular heart tissue	ND	ND	ND	ND	48.8 ± 5 mN/mm ²	n/a	Hibberd et al., 1982
Native human left ventricular heart tissue	ND	$1.5 \times 1.5 \times 12$ mm	ND	ND	3.7 mN/mm ² 22.8 mN/mm ²	174 bpm 72 bpm	Mulieri et al., 1992
Embryonic Chick V-CM	1×10^6	$15 \times 17 \times 4$ mm (at seeding)	1.15 ± 0.03 mN	0.09 ± 0.006 mN to 0.2 ± 0.01 mN	ND	72 bpm	Eschenhagen et al., 1997
Neonatal rat V-CM	1×10^6	D/T 8-10mm/1 mm	$0.1-0.3$ mN	$0.4 - 0.8$ mN	$0.5-1.1$ mN	ND	Zimmermann et al. 2002
hESC-CM hiPSC-CM	2×10^6	$20\text{mm} \times 0.5\text{mm}$	0.4 mN/mm ²	0.08 mN/mm ²	0.48 mN/mm ²	ND	Tulloch et al., 2011
Neonatal rat V-CM	500	Micro tissue <100 μ m thickness	8.25 ± 0.61 μ N	ND	ND	66 bpm	Boudou et al., 2012
hESC-CM	1×10^6	$7 \times 7 \times 1.2$ mm ²	ND	3.0 ± 1.1 mN 5.7 mN/cell	11.8 ± 4.5 mN/mm ²	ND	Zhang et al., 2013
hiPSC-CM	1×10^6	$100 - 200$ μ m	ND	ND	ND	55-80 bpm	Mathur et al., 2015

CM, cardiomyocytes; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell; ND, not determined; V, ventricular-type.