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Cardiac tissue engineering using human stem cell-derived cardiomyocytes for disease modeling and drug discovery

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Abstract

Cardiovascular disease (CVD) is the most prevalent health problem in the world, and the high mortality rate associated with irreversibly injured heart muscle motivates an urgent need for the development of novel therapies to treat damaged myocardium. Recently, human engineered cardiac tissues (hECT) have been created using cardiomyocytes derived from human embryonic stem cells and human induced pluripotent stem cells. Although a healthy adult phenotype remains elusive, such hECT display structural and functional properties that recapitulate key aspects of natural human myocardium, including dose related responses to compounds with known chronotropic, inotropic and arrhythmogenic effects. Thus, hECT offer the advantage over traditional *in vitro* culture models of providing a biomimetic 3D environment for the study of myocardial physiopathology, and may be used to generate preclinical models for the development and screening of therapies for CVD.

Introduction

Cardiovascular disease (CVD) is the most prevalent human health problem in the world. It is the leading cause of death in the United States, and currently claims more lives each year than cancer, chronic lower respiratory disease, and accidents combined. Despite the

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Conflict of interest

The author (s) have no conflict of interest to declare.

development of new treatments and decline of death rates attributable to CVD, the burden of disease and death remains high (1). Remarkable advances are being made toward the development of novel approaches for maintaining, restoring, or enhancing contractile function of diseased or damaged myocardium. Unfortunately, translation from bench to bedside has often been hampered by inconsistent results with adverse side effects. When such difficulties result in failed clinical trials (2) or market withdrawal of FDA approved pharmaceuticals (3), the costs in time, money, and patient lives are staggering. Such difficulties in translation are partly due to a missing link in available experimental methods; success in the Petri dish does not always translate to efficacy in animal models nor, ultimately, to humans.

A major breakthrough in CVD research has been the recent availability of cardiomyocytes (CM) derived from either human embryonic stem cells (hESC) or human induced pluripotent stem cells (hiPSC) (4, 5). Typically these differentiation processes result in a mixed cardiomyocyte phenotype, and efforts to sort them either with ventricular specific markers (6, 7), or transduction with genes expressed by specific cell phenotypes (8) offer promising approaches to obtaining homogenous cell populations. These offer attractive cell sources as implants for regenerative medicine (9). However, several challenges, including immune rejection (10) and teratoma formation (11), must be resolved before they are amenable for clinical use. Their current applications include *in vitro* models for studying cardiac physiology and development, offering an important species-specific advantage as a test tool for drug discovery and therapeutic innovation (12). Since hESC-CM and hiPSC-CM can contract spontaneously and respond to external stimuli (electrical and pharmacological), they are suitable for investigating cardiac electrophysiological responses to interventions at the cellular level, with high throughput screening facilitated by optical mapping (5, 13) and microelectrode array technologies (14, 15).

However, establishing an *in vitro* model for CVD involves several additional considerations. Cell responsiveness to geometry can influence growth and differentiation, possibly related to the distribution of cytoskeletal tension resulting from cell-cell and cell-substrate interactions (16). Mechanosensing of the microenvironment is evidenced by differences in cytoskeletal organization of cells grown in 2D vs. 3D environments (17). Cells are also responsive to changes in the mechanical properties of the culture substrate and extracellular matrix stiffness, which can influence their differentiation (18), and function, including the percentage of beating cardiomyocytes (19) and their contractile force (20). *In vitro* models need to move away from the standard culture conditions toward a biomimetic 3D system with controlled mechanical properties and force measurement capabilities. Meanwhile, *in vivo*, both large and small animal models have been designed for the study of cardiovascular disease, but have limited pathophysiological similarity to humans, are expensive, and are often too complex to isolate specific mechanisms. As an alternative, cardiac tissue engineering techniques may be useful to generate preclinical models of CVD.

Cardiac Tissue Engineering

There have been significant advances toward the long-term goal of developing a cardiac muscle patch for surgical implantation; however, significant technical and regulatory

obstacles to clinical applicability still remain (21). Meanwhile, interest in the use of engineered cardiac tissues (ECT) as *in vitro* models for developmental biology studies and therapeutic screening has expanded, and novel methods have been introduced for this purpose (22).

ECTs vary in their format and cell composition, depending on their ultimate application. Early examples of ECTs included strips created with cardiomyocytes from chicken embryo ventricles (23), and neonatal rat cardiomyocytes (NRCM) (24), or tissue loops that could undergo mechanical stretch (25), and be used as surgical grafts by stacking multiple tissues in combination (26). Cardiac patches can be created by seeding cells on dishes with temperature sensitive coatings (27), which yields an intact, matrix-free cell sheet to be used as a graft (28). The organization and function of these patches has been enhanced by applying electrical field stimulation (29) and interstitial flow of culture media (30). The aforementioned ECT depended on diffusion for gas and nutrient exchange, therefore their size was restricted to thin layers. To overcome this limitation, vascularized ECTs have been developed by seeding NRCM within a chamber surrounding an arteriovenous loop within the groin of a rat, which developed into a complex beating tissue several millimeters thick surrounding the native blood vessels (31). Despite numerous important developments, these studies focused on animal cell sources, which demonstrate species-specific responses that cannot always be generalized to human myocardium.

Recent progress in human cardiac tissue engineering

Development of human engineered cardiac tissues (hECT) first requires an appropriate source of human cardiomyocytes, as illustrated in Figure 1. Mesenchymal stem cells are a convenient source of human cells but they have not been reliably differentiated into functional CMs. Isolation of cardiomyocytes from patient heart biopsy is challenging and provides only a limited number of cells that cannot be expanded *in vitro*, and have a very short term viability lasting less than a week in culture. Perhaps the most promising cell sources for hECT have been the improved methods for differentiating hESCs and hiPSCs to human cardiomyocytes (5). Indeed, the development of cardiac tissue constructs from human pluripotent stem cell derived cardiomyoctyes is a relatively recent achievement. Investigators have placed cells onto matrices as patches for graft purposes (32, 33). However, such configurations are not entirely suitable for *in vitro* screening because they are not amenable to contractile function testing. The development of new treatments for heart disease would greatly benefit from human cardiac muscle created in the tissue culture laboratory, with controlled biological complexity and long-term viability, for *in vitro* monitoring of contractile function and high-throughput screening of novel therapies. Lately, there have been important developments in this area, which are summarized in the following paragraphs.

Tulloch et al. (34) first reported the creation of hECT from human pluripotent stem cell derived cardiomyocytes embedded in a collagen matrix, requiring at least 2 million cells per 100 μL for a construct 20 mm in length. With cell sources including hESC-CM and hiPSC-CM, these hECTs exhibited a positive force-length relationship, demonstrating the Frank-Starling mechanism observed in natural myocardium. Application of mechanical stress

induced features of early cardiomyocyte development within hECTs, including alignment, proliferation and hypertrophy. Static and cyclic mechanical stress of hECTs enhanced alignment of cardiomyocytes and extracellular matrix architecture when compared to unstressed and 2D culture systems; cyclic stress induced proliferation and hypertrophy of hESC- and hiPSC-CMs. These observations demonstrate the ability of such hECT models to recreate early developmental responses to external mechanical stimuli, which could be used to study human cardiac growth.

Schaaf et al. also reported the creation of hECTs using hESC-CMs and a mixture of bovine fibrinogen and thrombin (35). These hECTs exhibited beat to beat variability when exposed to increasing dosages of known proarrhythmic compounds, with threshold concentrations similar to hERG channel assay results, thus supporting the proposed use of hECT as a tool for preclinical toxicology. Cardiomyocytes within the ECT exhibited molecular and structural features indicative of phenotypic maturation compared to cardiomyocytes from age matched embryoid bodies (EBs); evidence included upregulation of mRNA expression levels of beta-myosin heavy chain (β-MHC), and while the trends toward increasing expression of other cardiac specific genes were not statistically significant, cardiomyocytes in the ECT were aligned and showed better sarcomeric organization. Thus, it was demonstrated that the 3D culture environment of hECT was more favorable than EBs for inducing cardiomyocyte maturation, and the sensitivity to proarrhythmic compounds supports their development for drug screening applications.

One limitation of previous studies is that the differentiated cells used to create hECTs comprise a population in which 50% or more are of an unspecified non-myocyte lineage, and the relative benefits or detriments of a mixed cell population remain poorly understood (36). In our laboratory, functional hECT have been created from a hESC-CM population that was >90% positive for expression of cardiomyocyte specific markers cardiac troponin-T (TNNT2) and α-sarcomeric actinin (ACTN2). With a focus on thorough characterization using multiple platforms, it was observed that these hECTs display functional parameters that are comparable to key aspects of natural human myocardium in several ways (unpublished data): they beat spontaneously at a rate of about 70 bpm; they have a 245-ms action potential duration (APD) that shortens at higher pacing frequencies, eventually losing 1:1 capture due to cardiac refractoriness; they have a functional Frank-Starling mechanism, and generate maximum isometric twitch forces of 660 μN, approaching values in newborn human myocardium; and known pharmacologic agents elicit expected chronotropic (isoproterenol) and inotropic responses (external calcium load, and verapamil), with half-maximal concentrations similar to human myocardium. Adenoviral-mediated gene transfer was also found to be effective at various time points in hECT culture, possibly opening new avenues for developing and testing gene therapies for heart disease.

Despite the rather immature cardiac phenotype, these 3D hECT represent critical advances in cardiac tissue engineering for human applications, promising a more natural *in vitro* model to enhance pre-clinical testing.

Functional assessment of engineered cardiac tissues

Quantitative evaluation of the response to pharmacological interventions is an important aspect of cardiac tissue physiology; it is an aid in understanding cellular mechanisms and generates data that is relevant for toxicology screening and drug discovery applications. Their effect on the engineered tissue can be assessed as a resulting change in chronotropic, inotropic or arrhythmogenic properties. A variety of platforms have been described for the assessment of a range of functional characteristics of the ECT. However, although some are more attractive for their ease of use or commercial availability, they each are accompanied by limitations in the type of measurements that can be obtained, indicating a need for new platforms designed specifically for hECT applications.

Physiologic muscle bath.

The muscle bath system has been traditionally used for *in vitro* physiology and pharmacology studies on myocardium (37). A thin sample of cardiac tissue (e.g., right ventricular trabeculae) is maintained under controlled temperature conditions, and bathed in a circulating buffer solution aerated with 95% oxygen and 5% CO_2. Using miniature hooks or tweezers, the tissue preparation is mounted to a high-sensitivity force transducer on one end, and to a micromanipulator or stepper motor on the other, allowing continuous force measurement with precise control of the tissue length. Experiments can be performed with or without electrical stimulation by means of electrodes connected to the mounting apparatus or applied directly to the tissue, and force measurements can be obtained under isometric or stretch-controlled conditions. Tissue viability can be maintained for hours or even days, providing a more physiologic environment than isolated cell cultures from the same tissue would allow. Muscle samples from different patient populations or different animal models can be studied under matched conditions to facilitate comparison and elucidate underlying mechanisms of the response. Of particular relevance for cardiac physiology are the responses to stretch and beating rate, where healthy human myocardium is characterized by positive force-length and force-frequency relationships. A variety of interventions can then be assessed, such as pharmacology experiments where drug response curves are generated. The physiologic muscle bath system is a well-established technique that has played a prominent role in our current understanding of myocardial physiology and pharmacology, and is regarded as the gold standard for muscle function testing, providing accurate isometric and length controlled force measurements. However, limitations of the method include that it is not applicable for high throughput screening, sterility is compromised and the ends of the tissue sample are damaged by the mounting apparatus precluding repeated longitudinal studies, and viability of the tissue under testing conditions is limited to 48 hours (37, 38).

Optical tracking of area deformation/shortening.

Optical monitoring can be used for measuring changes in cell or tissue area. Visual evidence of fractional area change of engineered cardiac constructs is used as a measure of contraction amplitude and strain (29), but this approach does not provide an actual value of the force generated by the tissue, and is clearly influenced by the underlying substrate or matrix stiffness.

Optical tracking of integrated elastomer end-posts.

The custom polydimethylsiloxane (PDMS) culture device with integrated flexible end-posts is a system that, unlike the physiologic muscle bath, maintains long-term sterility and viability, allowing repetitive measures for longitudinal studies (39). Similar in design to several comparable systems (40–43), the PDMS device is used both for creating and culturing hECT and for functional testing. The flexible elastomer end-posts bend with each tissue contraction, which can be optically tracked using a high-speed camera. Because the ECT remain attached to the culture device during testing, damage from tissue handling is avoided and long-term sterility is maintained. Reliable measurements of beating frequency and electrical pacing response can be obtained with this system, and using a simple beam bending equation, measurements of post deflection can be used to calculate twitch force and stress generated by the attached hECT. The main disadvantages of the PDMS culture device are related to the lack of length control: 1) the test is not performed under isometric conditions, such that diastolic properties may inadvertently affect systolic performance due to the Frank-Starling mechanism, complicating interpretation of measured twitch forces; 2) it is not possible to determine L_{max} (the length at which the hECT generates its greatest value of force) and specify a physiologically relevant length for testing contractile function, making it difficult to compare absolute values of twitch force between non-isometric studies. Therefore, until a modified elastomer system is developed to overcome these length-control issues, both the elastomer device and the physiologic muscle bath may be required to offer complementary techniques to ensure accurate and reliable testing of hECT contractile function for therapeutic development and screening applications.

Optical tracking of muscular thin films.

Muscular thin film (MTF) technology consists of flexible 2-D sheets of PDMS which are micropatterned with extracellular matrix protein and provide substrates for the growth of cardiac muscle monolayers from NRCMs (44) or progenitor-derived CMs (45). The film can then be cut into convenient shapes that undergo 3-D curling deformations that reflect the contractility of the adherent CMs. The MTF is clamped on one end, and set into a custom-built organ bath with an external field stimulator. Digital video of MTF contraction is recorded and converted to a binary skeleton representation, then the average radius of curvature is used to calculate contractile stress. MTF technology requires far fewer CMs than are needed to create hECTs, but the 2D monolayer configuration remains fundamentally different from the natural 3D cardiac microenvironment.

Optical mapping of impulse propagation.

Using tissues pre-treated with voltage- or calcium-sensitive fluorescent dyes, optical mapping of impulse propagation in response to point stimulation yields measurements of calcium transients, transmembrane voltage, maximum capture rate, conduction velocities, and APD. Originally developed for mapping natural cardiac tissue samples, these techniques have been successfully used with 3-D tissue cords composed of neonatal rat ventricular myocytes with a central bridge consisting of transfected excitable HEK 293 cells and also from 3-D cardiac tissue patches created with mouse embryonic stem cell-cardiomyocytes/fibroblasts and mouse embryonic stem cell-cardiovascular progenitor cells (36, 46). Using a

custom made optical mapping system (47), we have obtained recordings of maximum capture rate and APD at different pacing cycle lengths (unpublished data). The application of these electrophysiology recordings to hECT will generate data for the assessment of ion channel activity and sensitivity to pharmacologic agents with proarrhyhthmogenic potential. The main limitations are that the required fluorescent dyes have cytotoxic side effects, and the mapping apparatus does not provide a sterile environment, precluding repeated monitoring in longitudinal studies.

Optical tracking of fluorescent beads on microfabricated tissue gauges.

For high throughput force measurements, micro-cardiac tissues have been created with NRCM on PDMS arrays of miniature tissue gauges (μTUG) microfabricated using soft lithography and microelectromechanical systems (MEMS) technologies adapted from the semiconductor industry (48). The concept is similar to the PDMS end-post technology described above, but the dimensions are reduced to 800-μm long wells with cantilevers just 250 μm in height. For quantifying microtissue forces, brightfield and fluorescence videomicroscopy is used to track the displacement of fluorescent microbeads integrated into the cantilever tip. Miniaturization allows multiple samples to be monitored simultaneously, and requires a very low number of cells compared to other methods; 1 million cells yield approximately 200 micro-cardiac tissues with a final diameter of 50–70 μm (48, 49). This is advantageous for applications in which cell availability is limited, as with CMs created from hiPSCs. However, an ability to deliver different agents to individual micro-tissues within the array is still under development, and these devices are also not length controlled, leading to similar limitations as with the PDMS systems above.

Langendorff perfused heart system.

The Langendorff isolated perfused heart is the only system that goes beyond force measurements and can provide actual pressure-volume recordings, with direct relevance to clinical assessment of cardiac pump function. However, viability is limited to a few hours to one day at most with natural hearts (50). It also requires a unique type of engineered cardiac tissue having the geometry of a cardiac chamber (51–53), for which the preparation is rather involved and has not yet been implemented for high throughput applications.

Modeling heart disease in 3D engineered tissues

Engineered cardiac tissues have been shown to exhibit structural and molecular characteristics and electromechanical function similar to immature, but otherwise reasonably healthy, natural myocardium. Therefore, from the perspective of drug discovery applications, an important capability is to model impaired conditions representative of specific heart diseases targeted for therapeutic development. With this in mind, a number of cardiomyopathies have already been modeled using stem cell and tissue engineering approaches, as summarized below.

Myocardial fibrosis model.

To model the extracellular changes leading to fibrosis in post-infarction scar tissue, it has been shown that modifying the cross-linking properties of collagen can simulate scar tissue

formation (54). Scar formation following myocardial infarction introduces an arrhythmogenic substrate (55); therefore, the creation of engineered tissues with a fibrotic-like matrix could be used to evaluate the effects of wound healing and regenerative therapies on electrophysiology in a model of post-MI myocardium.

Another process affecting the extracellular matrix is age-related collagen cross-linking, which damages collagen fibrils and can involve enzymatic and nonenzymatic processes. A simplified *in vitro* model of nonezymatic-glycation used adult rat cardiac fibroblasts seeded on collagen gels pretreated or postreated with sodium nitrate (56). It was observed that the nonezymatic nitrate modification of collagen impaired cell-mediated remodeling and mechanical deformability of the tissue constructs. The authors noted that such a simplified system can be exposed to supraphysiologic conditions not possible *in vivo*, in order to accelerate reactions and expedite results (56). More recent studies on collagen cross-linking further confirm the concept of nonezymatic glycation and the role of *in vitro* models to modulate and analyze the properties of collagen (57). The use of such approaches in hECTs is relevant to the field of drug discovery because many common sources of nitrogen oxide, such as smoking and inflammation, are related to coronary artery disease and also to diabetic cardiomyopathy, both of which would benefit from development of improved therapeutic strategies.

Cryoinjury induced myocardial infarction model.

It is straightforward to create a global ischemia model by changing culture conditions to a low oxygen environment (58). While this reproduces some aspects of the acute injury, it is challenging to implement locally and does not recapitulate the chronic post infarct injury setting. Therefore, the cryoinfarct technique used in small animal models of myocardial infarction (59) was adapted for use with engineered cardiac tissues. ECTs were created from NRCMs in a collagen matrix and allowed to mature for several days. Then, after incubation with live/dead stain, the ECTs were subjected to cryoinjury by placement of a frozen steel dowel pin previously immersed in liquid nitrogen onto the middle third of the tissue length; fluorescent microscopy 20 minutes later confirmed the presence of dead cells in a well-defined region of injury with live cells on either side (60), yielding a model of localized chronic myocardial infarct that is well suited to studying regional function in response to various wound healing and regeneration strategies.

Dilated cardiomyopathy model.

Dilated cardiomyopathy and hypertrophy are adaptive compensatory mechanisms to restore left ventricular systolic performance in the presence of abnormal hemodynamic load. In these adaptive processes, wall stress has a major impact on cardiac tissue function, and the clinical relevance of this adaptation leading to heart failure and death has prompted experiments to identify targets to prevent left ventricular remodeling and altered myocardial contractile properties (61). These adaptive mechanisms have been described in animal models (62) and human donor hearts (63). As an *in vitro* model, 3-D cardiac organoid chambers created with NRCMs were able to emulate aspects of dilated cardiomyopathy, such that under a given pressure load, larger-diameter chambers experienced elevated wall stress and generated lower developed pressures compared to smaller chambers composed of

the same cells (51). Although many complexities of the heart failure disease process are neglected in such a model, it does permit decoupling of the effects of geometric remodeling from underlying changes in intrinsic myocyte contractility in a way that is not readily achieved in animal models of the disease. Such engineered cardiac organoids can thereby serve to screen therapies that specifically target one or the other of these underlying factors.

LEOPARD syndrome model.

The use of hiPSC–CMs uniquely allows investigation of cardiac diseases from patients who are carriers of point mutation abnormalities. For example, LEOPARD syndrome (named for its symptoms of lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary valve stenosis, abnormal genitalia, retardation of growth and sensorineural deafness) is an autosomal dominant disorder often with a missense T468M or Y279C mutation in the protein tyrosine phosphatase non-receptor type (PTPN)11 gene coding for the sarcoma (SRC) homology domain-containing protein tyrosine phosphatase (SHP)2 (64). In 2010, Lemischka and coworkers derived the first hiPSCs from fibroblasts of LEOPARD syndrome patients possessing the T468M mutation (65). The resulting diseased hiPSC-derived CMs displayed increased median cell area, increased sarcomeric organization and preferential nuclear localization of nuclear factor of activated T cells (NFAT)C4 relative to control cells, which are all in agreement with the cardiac hypertrophic phenotype often observed in LEOPARD syndrome patients. These effects were linked to a perturbation of the Ras-mitogen-activated protein kinase (MAPK) signal transduction pathway. Although one major symptom of this disease is the presentation of abnormal ECG, the electrophysiology of the derived CMs was not investigated; however, with the existence of these cells, such studies are now possible. Using such cells to create human ECTs would further allow investigation of contractile function, and targeted gene therapy to resolve the point mutation could also be explored using adenoviral-mediated transduction as described above. Other hiPSC-CM disease models that have been developed include long QT related cardiomyopathies. These are described in detail in our accompanying review.

Outlook for engineered tissue models of heart disease

hECT as a disease model.

Availability of mature human cardiomyocytes is severely limited, and functional engineered cardiac tissues have never been successfully created from adult cardiomyocytes. Therefore, although pluripotent stem cell derived cardiomyocytes represent an early developmental phenotype, they currently offer the best cell source for creating functional human ECTs. The immature hECT phenotype clearly limits the translation of results to adult human myocardium, since specific receptors and pathways required for mature cardiomyocytes to elicit a drug response can be deficient or absent in immature cells (66). Nevertheless, the early examples of hECTs described above are a significant step towards the development of an *in vitro* model that resembles natural human myocardium to fill a longstanding gap between traditional *in vitro* cell cultures and *in vivo* preclinical testing methods. Furthermore, with the creation of hECT using hiPSC-CMs, the response of a patient's surrogate cardiac tissue to different pharmacologic interventions could be used to identify possible cardio-toxic effects, allowing truly personalized drug screening.

A limitation of hECTs for modeling disease is that the isolated tissue lacks interaction with humoral, metabolic, neurogenic and vascular systems that impact CVD. One disadvantage is that it is oversimplified; on the other hand, because hECT is a living *in vitro* system, it allows studying multisystem interactions in a controlled way by selectively adding cellular, molecular, or serological components. Another limitation is that hESC-CMs used to create hECTs have exhibited phenotypic similarities to immature cardiomyocytes. Although various strategies have been proposed to induce hESC-CM maturation (8), efficacy remains incomplete. For surgical implantation applications of hECTs, this represents a major detriment. However, for disease modeling and drug discovery applications, hECTs composed of immature cardiomyocytes could arguably be more informative than healthy adult-like hECTs for modeling pathologies such as heart failure, in which cardiomyocytes are thought to reinitiate a fetal gene program (67).

hECTs also offer a number of advantages, including long term viability in culture, which are not possible with isolated natural heart tissue. Species-specific human disease responses should be detectable using hECTs created from hESC- or hiPSC-CMs. Furthermore, hECTs can be created from hiPSC-CM from patients with specific genetic abnormalities, providing a more biomimetic 3D culture system that could be amenable to examining disease mechanisms in patient-specific cardiac muscle. Therefore, despite simplifications of the hECT, there is a strong rationale for continued development as *in vitro* disease models.

hECT for drug screening.

Numerous factors must be considered in the design of a drug screening model, including: species, age of development, gender, perfusion rate and time, dosages to be tested, sensitivity, reproducibility of results, ease of access (ideally inexpensive and not technically challenging), and large scale capabilities (highly efficient, providing multiple tests per compound for a large number of compounds). It should permit a wide dose/concentration range to be tested. The outcome variables need to be well defined since these can vary amongst models (e.g., QT prolongation, APD variation, and instability or beat to beat variability can all represent proarrhythmia).

Considering all of these, the industry standard for screening pro-arrhythmic drugs includes hERG channel activity in HEK 293 cells, APD in dog or rabbit Purkinje fibers, and QT interval and rhythm in instrumented dogs, based on FDA and EMA recommendations for preclinical cardiotoxicity tests (35). Of note, these cells are of noncardiac or non human origin and are therefore subject to species-specific differences (14), primary cell sources are difficult to standardize, and animal models are not suitable for large scale screening. Although ECTs offer advantages in all three of these areas, further characterization is required to comprehensively identify the limits within which hECTs can be reliably used for pre-clinical testing. This includes a better understanding of the cardiac phenotype, and expanded testing of the number and variety of drugs with known cardiac effects in humans (68).

Moving towards human cell sources, and better yet, surrogate human tissue, is an important means to overcome species specific and standardization challenges, generating models that will better demonstrate the pharmacologic response that would be elicited in natural human

myocardium. High throughput still remains a challenge with hECT; while micro-cardiac tissues allow for high throughput creation of ECTs (48), the number of testing conditions that can be simultaneously monitored remains limited. Other innovations that would be beneficial are voltage sensitive reporters that are not toxic for long term electrophysiological monitoring, as well as length control for contractile function testing.

Conclusion

The field of cardiac tissue engineering has made tremendous progress toward creating functional surrogates that mimic natural myocardium for a range of applications from surgical implantation, to basic science, to drug discovery and toxicology screening applications. The recent success creating ECTs from human derived cell sources (including hESCs and hiPSCs) represents a major breakthrough by providing a species-specific model of human heart muscle. A number of methods have been used for monitoring function of ECTs, and although a truly high-throughput assay has not yet been achieved, existing methods have revealed a number of similarities, as well as some important differences, between hECTs and natural human myocardium. In particular, hECTs exhibit phenotypic characteristics similar to immature or failing heart muscle, motivating efforts to induce directed maturation. Improvements in the yield and purity of cardiomyocytes derived from human pluripotent stem cells will further assist in achieving high throughput applications for these hECTs. Finally, a variety of cardiac disease models can be created using human tissue engineering. The future of this research remains highly promising, and the development of living, human engineered cardiac tissues designed for high-throughput *in vitro* evaluation of candidate interventions on patient-specific cardiac function has the potential to fundamentally transform the fields of disease modeling and drug discovery.

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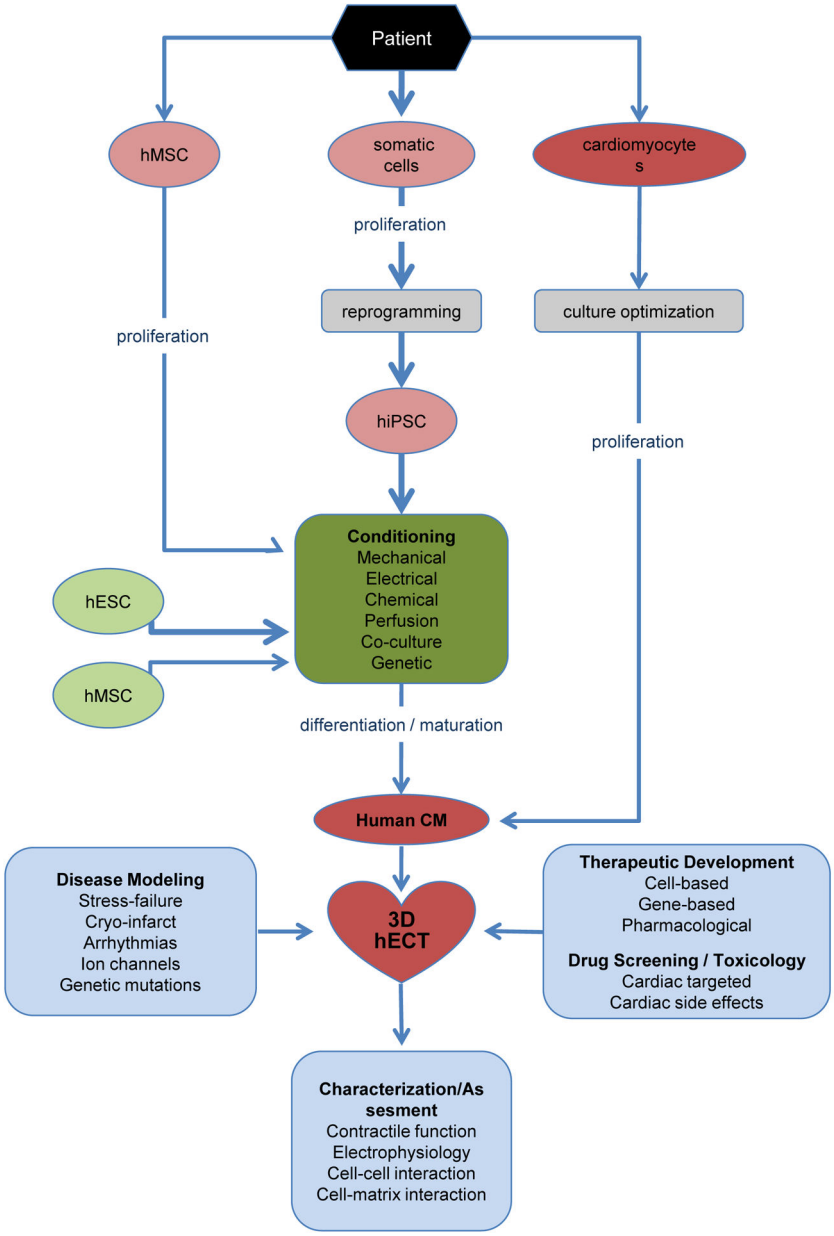

Figure 1. Paths toward the development of patient-specific *in vitro* 3D human engineered cardiac tissues (hECT) for *in vitro* disease modeling and drug discovery.

Allogeneic cells (light green), including human embryonic stem cells (hESC) and mesenchymal stem cells (hMSC), are excellent resources even though they are not patient-specific; hESC-derived cardiomyocytes (CMs) have been the main source for creating hECT to date. Adult cardiomyocytes from patients (red) are very limited in supply and are quiescent, thus requiring new culture optimization approaches to induce *in vitro* proliferation and obtain cell numbers sufficient for creating hECT. Autologous cell sources (pink) include bone marrow hMSCs and somatic cells reprogrammed into induced pluripotent stem cells (hiPSC), which can then be differentiated into cardiomyocytes. The hCMs thus obtained typically exhibit characteristics of the immature heart; therefore, conditioning processes (green) must be optimized to induce phenotypic maturation of engineered cardiac cells and

tissues. The procedures and applications for which such hECT can be implemented are multifarious (blue), including a) characterization and functional assessment to investigate cardiac development and physio-pathological processes, b) modification of hECT as disease models for specific cardiac anomalies, and c) utilization of hECT to develop novel therapeutic approaches, and for drug screening and toxicology applications.