Human Induced Pluripotent Stem Cell–Derived Cardiomyocytes
Insights Into Molecular, Cellular, and Functional Phenotypes

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Abstract: Disease models are essential for understanding cardiovascular disease pathogenesis and developing new therapeutics. The human induced pluripotent stem cell (iPSC) technology has generated significant enthusiasm for its potential application in basic and translational cardiac research. Patient-specific iPSC-derived cardiomyocytes offer an attractive experimental platform to model cardiovascular diseases, study the earliest stages of human development, accelerate predictive drug toxicology tests, and advance potential regenerative therapies. Harnessing the power of iPSC-derived cardiomyocytes could eliminate confounding species-specific and interpersonal variations and ultimately pave the way for the development of personalized medicine for cardiovascular diseases. However, the predictive power of iPSC-derived cardiomyocytes as a valuable model is contingent on comprehensive and rigorous molecular and functional characterization. (Circ Res. 2015;117:80-88. DOI: 10.1161/CIRCRESAHA.117.305365.)

Key Words: cardiovascular diseases ■ cardiovascular disease modeling ■ induced pluripotent stem cells ■ myocytes, cardiac ■ precision medicine

Induced Pluripotent Stem Cell–Derived Cardiomyocytes: A New and Versatile Human In Vitro Cardiomyocyte Model

Recent advances in genomics and molecular medicine promise to revolutionize human health by enabling more precise prediction, prevention, and treatment of cardiovascular diseases on an individual level.1 This precision medicine approach is based on the ability to diagnose and stratify patients into different treatment groups by correlating a patient’s genotype with their cellular phenotype, and uncover how the genetic differences among people could influence their responses to therapies. However, realizing this potential requires the development of accurate disease models. Models that recapitulate individual patients’ disease at the molecular and cellular level could lead to a better understanding of the disease progression and pathogenesis and ultimately enable the prediction of individual patient’s responses to targeted treatments.

Disease models have been and will continue to be instrumental in providing important insights into the molecular basis of cardiovascular development and disease.2 The knowledge gained from studying transgenic animals and transformed cell lines has already been successfully applied to understand human cardiovascular disease. Nevertheless, this translation would be significantly strengthened by the availability of patient-specific in vitro models. Human-based models are particularly important for cardiovascular research because the physiology of animal models is different from human cardiomyocytes. Particularly, considerable differences exist between cardiomyocytes from small animal models and human cardiomyocytes, including beating rates, energetics, Ca2+ cycling, myofilament composition, expression of key ion channels, and cellular electrophysiology. These differences in physiology are substantially less between humans and large animal models such as nonhuman primates, pigs, and dogs.3

The recent advent of the human induced pluripotent stem cell (iPSC) technology,4 and an increasingly refined capacity to differentiate iPSCs into disease-relevant cell types such as cardiomyocytes (iPSC-CMs),5 provides an unprecedented opportunity for the generation of human patient-specific cells for use in disease modeling, personalized drug screening, and regenerative approaches toward precision medicine.6,7 Implementation of this unique and clinically relevant model system presents a significant advantage in cardiovascular research as it can circumvent complications in translating data from models across different species and biological characteristics.

iPSC-CMs offer several advantages over current in vitro models such as immortalized cell lines, human cadaveric tissue, and primary cultures of nonhuman animal origin. First, the derivation of iPSC-CMs is at most minimally invasive.
During the past few years, there has been considerable progress in the iPSC-CM technology and its contributions to cardiovascular research are already well recognized. For example, iPSC models have been recently used to describe cardiac channelopathies such as long-QT syndromes (LQT1, LQT2, LQT3/Brugada syndrome, and LQT8/ Timothy syndrome), catecholaminergic polymorphic ventricular tachycardia (CPVT), arrhythmogenic right ventricular dysplasia (ARVD), familial hypertrophic cardiomyopathy (HCM), and familial dilated cardiomyopathy (DCM). As the iPSC-CM technology continues to evolve, it will greatly facilitate the study of inherited and acquired cardiovascular diseases, infectious diseases, cardiovascular development, drug discovery, toxicology screening, and personalized cell therapy (Figure 1).

In this review article, we will highlight the current state of iPSC-CMs, focusing on their phenotype and function. We will discuss the molecular phenotypes, electrophysiological and calcium handling properties, and bioenergetics. We will also explore what the future may hold for their use in cardiovascular research, pharmacology, and regenerative medicine toward precision medicine.

**Generation of iPSC-CMs**

Studies with different model organisms have demonstrated that signaling pathways, such as activin/nodal/transforming growth factor-β, Wnt, and bone morphogenetic protein, play pivotal roles in establishing the cardiovascular system. By mimicking endogenous developmental signaling cues, direct differentiation methodologies for generating iPSC-CMs have been developed. Several permutations of growth factors and small molecules have recently been reported to improve reproducibility and efficiency of iPSC-CM differentiation protocols in both adherent and suspension cultures. Further purification of CMs from a mixed population of iPSC-derived cells can be accomplished by nongenetic methods, including cell-surface markers, mitochondria-specific dyes, fluorescent probes, and glucose deprivation. Although iPSC lines seem to respond differently to developmental signals because of the intrinsic differences in their genetic background, these differentiation protocols have been successfully applied to iPSCs derived from distinct sources of somatic cells and reprogramming methods. However, the resultant iPSC-CM population is a heterogeneous pool of atrial-, ventricular-, and nodal-like cells. As native atrial, nodal, and ventricular myocytes possess distinct molecular and functional properties, coaxing human iPSC differentiation toward specific cardiomyocyte subtypes remains challenging for the current methodologies. In this respect, recent reports suggest that pluripotent stem cells could be directed either to atrial- or to ventricular-like cardiomyocytes by modulating the retinoic acid and Wnt signaling pathways. However, the elucidation of the molecular mechanism(s) underlying the cardiomyocyte subtype specification would be essential to further refine the current differentiation protocols and improve our understanding of lineage-specific development.

**Molecular Profiling**

At the molecular level, the differentiation of iPSCs toward the cardiomyocyte lineage is orchestrated by the sequential expression of distinct sets of genes in specific stages in a pattern consistent with normal cardiac development: mesoderm formation (T and MIXL1), cardiogenic mesoderm (MESP1, ISL1, and KDR), cardiogenic progenitors (NXX2.5, GATA4, TBX5, MEF2C, and HAND1/2), and structural genes encoding for sarcomeric-related proteins of terminal differentiated cardiomyocytes (MYL2, MYL7, MYH6, and TNNT2). Gene expression analysis has revealed that normal iPSC-CMs and disease-specific iPSC-CMs expressed all the major cardiac ion channel genes found in the adult left ventricular cardiac tissue, including sodium channel (SCN5A), L-type calcium channels (CACNA1C and CACNA1D), and potassium channels ( KCNH2 and KCNQ1). Furthermore, iPSC-CMs express genes that encode critical components of the Ca2+ cycling machinery such as inositol trisphosphate receptor (IP3R3), ryanodine receptor 2 (RYR2), sarcoplasmic reticulum (SR) Ca2+-ATPase (SERCA2), calregulin (CALR), junctophilin 2 ( JPH2), phospholamban (PLN), sodium calcium exchanger (NCX1), and triadin (TRDN). However, their relative expression differs from that of the human adult ventricular tissue. Mitochondrial complexes I–V and genes involved in cholesterol metabolism (PRKAG1 and PRKAG2) as well as genes that confer protection against apoptotic and oxidative stress processes (BCL2L1 and SOD1, respectively) are also expressed in iPSC-CMs. Taken together, these studies demonstrated that the gene expression in iPSC-CMs closely mirrors the patterns observed in human cardiomyocytes (Figure 2).

From a disease modeling perspective, the faithful expression of disease-associated alleles is a prerequisite for proper manifestation of the disease phenotypes in iPSC-CMs. For example, mutations in genes encoding sarcomeric components, including TNNT2 and MYH7, have been implicated in the 2 most common forms of inherited cardiomyopathies: HCM and DCM. Mutations in the genes encoding potassium ( KCNQ1 and KCNH2), sodium ( SCN5A), and calcium (CACNA1C) channels are the most common cause of the LQT syndromes, which is usually inherited in an autosomal dominant manner. Recently, iPSC-CMs have been derived from patients...
harboring deleterious mutation that recapitulated key disease aspects of HCM, DCM, and LQT syndromes. Such models are currently being used to decipher the complex genotype-phenotype relationships and to determine disease effects of specific genetic variants.

Ultrastructural Features

To maximize the potential applications of iPSC-CMs in cardiovascular medicine, it is essential for these cells to recapitulate the ultrastructural properties of adult cardiomyocytes. It has been reported that early stage iPSC-CMs are small morphologically and exhibit an immature ultrastructure closely resembling that of fetal CMs (ie, absence of T-tubules and underdeveloped contractile machinery). However, on prolonged culture, there were significant improvements in myofibril alignment, density, and morphology showing adult-like appearance of Z-disks, A-bands, I-bands, and H-zones, although no clear M-bands or T-tubules were observed. Similarly, by combining bioengineering approaches with electric stimulation, Nunes et al developed a platform called biowire that enables the generation of iPSC-CMs with ultrastructural properties similar to those seen in the native CMs. The progressive lengthening of 3-dimensional iPSC-CM tissues that mimics heart growth during development further improved cell alignment and increased sarcomeric ultrastructural organization. Despite these advances, the contractile properties of iPSC-CMs and engineered tissues remain at rudimentary levels.

Electrophysiological Phenotypes and Ion Channel Function

Comprehensive analyses of the electrophysiological properties of iPSC-CMs have been reported. Based on the action potential (AP) phenotypes recorded in isolated cells, iPSC-CMs consist of a heterogeneous population categorized as atrial-, nodal-, or ventricular-like. However, cell culture conditions and differentiation protocols may influence these AP properties, possibly undermining the correctness of this classification. Although the direct electrophysiological comparison between iPSC-CMs and human adult CMs is challenging because of experimental discrepancies, tissue heterogeneity, and disease status, it is well documented that iPSC-CMs display mixed AP phenotypes characterized by relatively positive maximum diastolic potential and slower
Figure 2. Expression of key structural and functional genes in induced pluripotent stem cell–derived cardiomyocytes (iPSC-CMs).

**A.** Schematic of the major structural and functional features of iPSC-CMs. In adult CMs, on membrane depolarization a small amount of Ca$^{2+}$ influx induced by activation of voltage-dependent L-type Ca$^{2+}$ channels (CACNAC1) triggers the release of Ca$^{2+}$ from the sarcoplasmic reticulum (SR) through the ryanodine receptors (ryanodine receptor 2 [RYR2]), termed Ca$^{2+}$-induced Ca$^{2+}$-release mechanism. The released Ca$^{2+}$ ions diffuse through the cytosolic space and bind to troponin C (TNNC1), resulting in the release of inhibition induced by troponin I (TNNI3), which activates the sliding of thin and thick filaments, and lead to cardiac contraction. Recovery occurs as Ca$^{2+}$ is extruded by the Na$^{+}$/Ca$^{2+}$ exchanger (NCX1) and returned to the SR by the sarco(endo)plasmic Ca$^{2+}$-ATPase pumps on the nonjunctional region of the SR that are regulated by phospholamban (PLN). This process is conserved in iPSC-CMs, but major differences exist, such as a nascent SR, the presence of an inositol 1,4,5-trisphosphate–releasable Ca$^{2+}$ pool, and the complete absence of T-tubules. The genes encoding the major transmembrane ion channels involved in the generation of action potential are also shown.

**B.** Immunofluorescence staining of cardiac troponin T and α-sarcomeric actinin in iPSC-CMs.

**C.** Line-scan images and spontaneous Ca$^{2+}$ transients in iPSC-CMs. ACTC1 indicates actin, α, cardiac muscle 1; CACNA1C, calcium channel, voltage-dependent, L type, α1C subunit; IPTR3, inositol 1,4,5-trisphosphate receptor, type 3; KCNQ1, potassium voltage-gated channel, KQT-like subfamily, member 1; MYBPC3, myosin binding protein C; MYH6, myosin, heavy chain 6, α; MYH7, myosin, heavy chain 7, β; MYL2, myosin, light chain 2; MYL3, myosin, light chain 3; SCN5A, sodium channel, voltage-gated, type V, α1 subunit; SERCA2, SR Ca$^{2+}$-ATPase 2; TNNI3, troponin I type 3; TNNT2, troponin T type 2; TTN, titin; and TPM1, tropomyosin 1 (α).
upstroke velocity when compared with the human native counterparts.\textsuperscript{53} Moreover, recent studies suggest that the ventricular-like iPSC-CM subtype exhibits many key cardiac electrophysiological properties analogous to those of human CMs. The ventricular-like APs display properties of more mature human CMs, including a distinct plateau phase (phase 2) after which repolarization accelerates (phase 3), with AP durations that are within the normal range of the human electrocardiographic QT interval (Figure 3A).

Multiple ionic currents have been characterized in single iPSC-CMs, including the sodium (\(I_{\text{Na}}\)), the L- and T-type calcium (\(I_{\text{Ca,L}}\) and \(I_{\text{Ca,T}}\)), the hyperpolarization-activated pacemaker (\(I_{f}\)), the transient outward potassium (\(I_{\text{Ko}}\)), the inward rectifier potassium (\(I_{\text{Kr}}\)), and the rapid and slow activating components of the delayed rectifier potassium currents (\(I_{\text{K1}}\) and \(I_{\text{Ks}}\), respectively; Figure 3B). However, the functional properties of additional currents, such as the ATP-sensitive K\(^+\) current (\(I_{\text{K,ATP}}\)) and the Na\(^+-\)Ca\(^+\) exchange current (\(I_{\text{NCX}}\)), have not yet been reported in iPSC-CMs, whereas the atrial-selective ion current, acetylcholine-activated K\(^+\) (\(I_{\text{K,ACh}}\)), has recently been reported in human embryonic stem cell-derived atrial-like CMs.\textsuperscript{56} We briefly summarize some of these major channels below:

\(I_{\text{Na}}\): iPSC-CMs have prominent Na\(^+\) currents with activation and inactivation gating characteristics that are analogous to those of native human ventricular CMs,\textsuperscript{14,53}

\(I_{\text{Ca,L}}\) and \(I_{\text{Ca,T}}\): The activation and inactivation gating properties of \(I_{\text{Ca}}\) in iPSC-CMs are similar to those obtained from human ventricular cardiomyocytes.\textsuperscript{15,53} By contrast, although the \(I_{\text{Ca,T}}\) current has been found in a subset of cells, its properties have not been defined.\textsuperscript{56}

\(I_{f}\): In ventricular-like iPSC-CMs, the presence of hyperpolarization-activated \(I_{f}\) promotes phase 4 depolarization and thus contribute to automaticity.\textsuperscript{53}

\(I_{\text{K1}}\): Three K\(^+\) currents (\(I_{\text{Ko}}, I_{\text{Kr}}\), and \(I_{\text{K1}}\)) have been recorded in iPSC-CMs with maximum densities and activation properties comparable with the values reported for human cardiac myocytes.\textsuperscript{33} By contrast, the density of \(I_{\text{Kr}}\) is either absent or significantly smaller than that reported for native ventricular CMs. Importantly, \(I_{\text{Kr}}\) contributes to repolarization of the cardiac AP, and block of \(I_{\text{Kr}}\) prolongs the ventricular AP, which is manifested by QT prolongation on the surface ECG. Prolongation of the AP and associated increased QT interval can lead to early afterdepolarizations on the cellular level and trigger the ventricular arrhythmia Torsades de pointes. Notably, by measuring AP duration and quantifying drug-induced arrhythmias, such as early afterdepolarizations and delayed afterdepolarizations, drug-induced cardiotoxicity profiles for healthy subjects, LQT syndrome, HCM, and DCM patients were recapitulated at the single cell level using the iPSC-CM technology.\textsuperscript{38,53} Interestingly, the iPSC-CMs exhibited distinct responses to known cardiotoxic drugs when derived from healthy versus diseased individuals, suggesting that adverse drug responses could be accurately predicted in individual patients. This raises the prospect of proarrhythmic drugs being readily identified early in a development program, and those individuals at high risk for proarrhythmia could be identified before deleterious drug exposures. The use of iPSC-CMs in screening for proarrhythmic potential of current and new drug entities in conjunction with in silico modeling is a major focus of the Food and Drug Administration Comprehensive In Vitro Proarrhythmia Assay initiative.\textsuperscript{54}

In summary, multiple voltage-gated ion channels are similarly present in iPSC-CMs and adult CMs leading to characteristic cardiac APs (Figure 3B). However, significant differences do exist, such as reduced inward rectifier K\(^+\) currents and the presence of prominent pacemaker currents. As a result, the iPSC-CMs exhibit spontaneous automaticity, which is not observed in healthy human ventricular cardiomyocytes. At the tissue level, these properties may be significantly altered when the cells are coupled in a functional syncytium and recent efforts
have been focused on using electric or mechanical stimulation that seems to promote electrophysiological maturation.46-50 Future studies are clearly needed to characterize in detail the electrophysiological properties of iPSC-CMs at both single-cell and tissue levels.

Excitation Contraction Coupling and Calcium Handling
Myocardial contraction and relaxation are coordinated on a beat-to-beat basis by the orchestrated cycling of calcium from the cytoplasm, SR, and the sarcomere through excitation–contraction coupling.39 Extensive characterization of spontaneous whole-cell [Ca2+]i transients in iPSC-CMs suggests the presence of functional excitation–contraction coupling that resembles the native myocardium.41 Specifically, it has been demonstrated that Ca2+ influx via the depolarization-activated L-type Ca2+ channels triggered a marked release of the SR Ca2+ stores via the Ca2+ sensitive ryanodine SR receptors, recapitulating the Ca2+-induced Ca2+-release phenomenon in iPSC-CMs.41,46 A key mechanism underlying excitation–contraction coupling.39 Of note, iPSC-CM cultured on microgrooved substrates displayed significantly improved Ca2+ cycling and more organized SR Ca2+ release in response to caffeine, suggesting that SR Ca2+ cycling properties can be influenced by culture conditions.40 In catecholaminergic polymorphic ventricular tachycardia, an inherited disease characterized by stress-induced ventricular arrhythmias, iPSC-CM–based modeling also supports the presence of functional SR and ryanodine receptor Ca2+ transients.37 However, the Ca2+ handling kinetics in iPSC-CMs seem to be relatively slow and characterized by a U-shaped wave, suggesting that iPSC-CMs have an immature Ca2+-induced Ca2+-release mechanism.60 Indeed, iPSC-CMs exhibit a poorly developed SR and absence of T-tubules that likely affect their Ca2+ handling properties.43,44

Metabolic Profile
Various studies have demonstrated that iPSC-CMs have a metabolic phenotype that resembles embryonic cardiomyocytes, which mostly rely on glycolysis for energy production instead of lipid oxidation as seen in adult ventricular myocytes. Strategies to facilitate metabolic maturation have been developed, including culturing iPSC-CMs with an adipogenic cocktail19 or glucose-free medium61 and tissue engineering approaches.62 These approaches were able to yield advanced metabolic phenotypes. The structure and function of iPSC-CMs can be further enhanced by prolonged culture and bioengineering approaches, but the factors and signaling pathways affecting maturation are incompletely understood. Diverse epigenetic processes, including long-noncoding RNA,64 microRNAs,64,65 chromatin and histone proteins,66,67 and DNA methylation,68,69 have emerged as critical modulators of cardiac gene expression in development and disease. Hence, future studies using high-throughput omic technologies63 will be essential to unravel the genetic and epigenetic mechanisms involved in shaping the phenotype of iPSC-CMs. A better understanding of the underlying mechanisms could potentially lead to the development of strategies to create cardiomyocytes with mature-like phenotypes. The functional maturation is indeed a desirable phenotype, but it is important to acknowledge that a phenotype resembling the adult cardiomyocytes might not be attainable in vitro cell culture conditions. It is also important to recognize that the relatively immature phenotype of the iPSC-CMs is not necessarily a disadvantage for certain applications. For example, immature cells could potentially be better suited for cell therapy applications, as they can become mature after integration into the host myocardium, as recently observed for embryonic stem cell–derived cardiomyocytes.70

With the launching of the Precision Medicine Initiative,1 the iPSC-based models of cardiovascular disease are well positioned to provide a powerful tool for studying genotype–phenotype association and for predicting individual patient responses to therapies. However, relating gene variations
among individuals to clinical phenotypes may not be straightforward. Ultimately, we will need to evaluate and validate the iPSC-CM technology using larger numbers of patient-specific cell lines. To this end, the availability of well-characterized, disease-relevant iPSC biobanks will be indispensable to assess their predictive power. The recent initiatives by the National Institutes of Health and the California Institute of Regenerative Medicine to establish state-of-the-art human iPSC repositories will address the increasing need for quality-controlled, disease-relevant, and research-grade iPSC lines. Eventually, these efforts will provide researchers across academia and industry with access to high-quality iPSC lines from diverse genetic backgrounds and cardiovascular diseases to conduct prospective studies to establish causal associations between genetic variations and drug response.

New technologies, including next-generation sequencing and nuclease-mediated genome editing, are rapidly advancing the application of the iPSC-CM technology toward future precision medicine approaches. Targeted gene editing using site-specific nucleases, such as zinc finger nucleases (ZNF), transcription activator–like effector nucleases (TALENs), and the clustered regularly interspaced short palindromic repeat/clustered regularly interspaced short palindromic repeat–associated protein 9 systems (CRISPR/Cas9), is a powerful tool that allows for reverse genetics, genome engineering, and targeted transgene integration experiments to be performed in a precise and predictable manner. Indeed, genes have been added into specific loci and gene mutations have been introduced or used to correct disease-causing mutations in iPSCs-based cardiovascular disease models in vitro. The most common application to date has been to correct mutations that cause monogenic cardiomyopathies, including DCM and BTHS, and long-QT syndrome. These studies have provided a proof of principle that the observed phenotypes are caused by specific mutations, suggesting that this approach could be used to uncover the underlying pathological disease mechanism. It should be noted, however, that gene editing with engineered nucleases is problematic. Significant challenges remain, including specificity and off-target effects, efficiency, selection of targeted sites, and delivery methods.

Overall, the iPSC-CMs present a new and rapidly developing technology with exciting applications, and with further refinements it could pave the way for the development of personalized medicine for cardiovascular diseases.

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Disclosures

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References


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