Induced pluripotent stem cells for cardiovascular disease: from product-focused disease modeling to process-focused disease discovery

Induced pluripotent stem (iPS) cell technology offers an unprecedented opportunity to study patient-specific disease. This biotechnology platform enables recapitulation of individualized disease signatures in a dish through differentiation of patient-derived iPS cells. Beyond disease modeling, the in vitro process of differentiation toward genuine patient tissue offers a blueprint to inform disease etiology and molecular pathogenesis. Here, we highlight recent advances in patient-specific cardiac disease modeling and outline the future promise of iPS cell-based disease discovery applications.

**Keywords:** cardiomyocyte • cardiomyopathy • channelopathy • disease discovery • disease modeling • disease pathogenesis • induced pluripotent-stem cell • regenerative medicine

**Background**

Induced pluripotent stem (iPS) cells are a bioengineered cell type derived via nuclear reprogramming of somatic tissue into a pluripotent state. First described in mice [1] and shortly thereafter in humans [2,3], iPS cells offer an unparalleled source of patient-specific, pluripotent stem cells that promise to advance next-generation diagnostic and clinical regenerative medicine [4,5]. While the scalability and safety of iPS cell-based clinical applications remain under investigation [6], these cells increasingly provide an opportunity to target human cardiac diseases in a dish [7]. In fact, there has been significant progress using iPS cells as a platform for in vitro disease modeling, including a growing number of examples of patient-specific models of channelopathies and cardiomyopathies [8–18]. The capacity of iPS cells to undergo differentiation into cardiac phenotypes enables the study of individualized disease processes in a highly controlled setting. Importantly, the proliferative nature of iPS cells provides an essentially unlimited pool of patient-specific cardiac progenitors and cardiomyocytes for investigation. Pharmacologic screens for novel therapeutic agents can now be conducted on functional human cardiomyocytes to serve as an individualized read-out of small molecule efficacy without risk of toxicity to the patient [19]. Here, we review the current progress in cardiac disease modeling applications and the future possibilities of cardiovascular disease discovery with patient-specific iPS cells.

**Disease modeling: defining cell-autonomous disease-in-a-dish**

As a benchmark to gauge the transformative potential of iPS cells, it is important to note that traditional disease diagnostic methods are typically linked to the pathophysiological context of the patient (Figure 1). Thus, clinical observations of disease are confounded by the mixture of disease-causing mechanisms and compensatory pathways. Without the ability to separate cause and effect, the current clinical paradigm may misconstrue compensatory mechanisms as contributors to disease etiology, or vice versa. However, through in vitro differentiation of iPS cells, we can now follow sequential cellular phenotypes from individual patients without the obstructive effects of surrounding physiology (Figure 1). Thus, iPS cell-based disease mod-
Disease modeling: generation of patient-specific induced pluripotent stem cells enables individualized recapitulation of disease signatures following differentiation into beating cardiomyocytes. Disease discovery: emphasis on the process of in vitro differentiation provides novel insight into early mechanisms of disease pathogenesis.

Table 1 highlights recent disease modeling studies that use patient-derived iPS cells to model cardiac diseases and emphasizes the characteristics of cell phenotypes that were studied in each model. In these studies, patient-specific cardiomyocytes have been identified by a variety of gene and protein expression profiles, including sarcomeric proteins (ACTN2, MYH6, MYH7, MYL2, MYL7, TNNT2, TNNI3, TTN), cardiac transcription factors (ISL1, HAND-1, NKKX2.5, GATA4, TBX5, NFATC4), calcium handling proteins (CACA1C, CACNB2, PLN, RYR2, CASQ2, FKBP1B, CALM, CALR, SERCA, TRDN, JCTN), potassium ion channels (KCNQ1, KCNH1, KCNJ2, KCND3, KCNA5, KCNJ5, KCNE1, KCNJ3, KCNJ11, KCHIP2, KCNA4, KCN2, HCN2, HCN4), sodium ion channels (SCN5A, SLC8A1), chloride channels (CLCN4), hormones (ANP) or other cardiomyocyte surface markers (ADRB1, ADRB2, CX43, VCA1M). It is important to note that all cardiovascular disease models highlighted herein have utilized contractile cardiomyocytes as the cell phenotype to recapitulate the signature of disease. While pure populations of iPS cell-derived cardiomyocytes remain difficult to efficiently and reliably generate via current methods of in vitro differentiation, the studies described below use a variety of cardiomyocyte markers to selectively study beating cardiac phenotypes in vitro.

iPS cell models of cardiac channelopathies

One of the first cardiac channelopathies modeled by iPS cells was long QT syndrome (LQTS), a disorder characterized by prolonged ventricular repolarization and increased propensity for polymorphic ventricular tachycardia [20]. Within 3 years of the initial description of human iPS cells [2,3], a patient-specific model of Type 1 LQTS (R190Q mutation in KCNQ1) was established [20]. In this study, dermal fibroblasts were isolated from related patients with LQTS1 and reprogrammed into iPS cells using retroviral vectors for OCT3/4, SOX2, KLF4 and c-MYC. Following nuclear reprogramming, patient-specific and healthy control iPS cells were differentiated into functional cardiomyocytes. Importantly, iPS cell-derived cardiomyocytes from LQTS1 patients recapitulated the electrophysiological signatures of disease (prolonged action potential duration and increased susceptibility to catecholaminergic arrhythmias) when compared with healthy controls. The power of this LQTS model is best realized in comparison to the limitations of established animal models of LQTS [16]. For example, differences in action potential characteristics, cardiomyocyte physiology and heart rate between mouse and human hearts limit the informative nature of LQTS mouse models [16]. Establishing a patient-specific, cell-autonomous model of LQTS1 has been a breakthrough for the field of cardiac channelopathy research and is driving new paradigms for the pharmaceutical industry.

Similar disease models have recently been established of LQTS1 [21,22], as well as LQTS2 (mutations in KCNHN2) [23–26] and LQTS3 (mutations in SCN5A) [27,28]. These studies have uniformly demonstrated that patient-specific iPS cell-derived cardiomyocytes can recapitulate disease electrophysiology. Specifically, prolonged action potential duration and increased propensity to drug-induced arrhythmias...
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<th>Disease classification</th>
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<th>Somatic origin</th>
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<th>Molecular markers</th>
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<td></td>
<td>Timothy syndrome</td>
<td>Dermal fibroblasts</td>
<td>Beating CMs</td>
<td>ACTN2, TNNI3, MYH7, ANP</td>
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<td>Cardiomyopathy</td>
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<td></td>
<td>HCM</td>
<td>Dermal fibroblasts</td>
<td>Beating CMs</td>
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<td></td>
<td>HCM</td>
<td>Dermal fibroblasts</td>
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<td>TNNT2, MYL2, MYL7, MYH6, MYH7, NFATC4, ANP</td>
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ARVC: Arrhythmogenic right ventricular cardiomyopathy; BTHS: Barth syndrome; CM: Cardiomyocyte; CPVT: Catecholaminergic polymorphic ventricular tachycardia; DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; JLNS: Jervell and Lange-Nielson syndrome; LQTS: Long QT syndrome.
such as early after depolarizations have been observed in LQTS iPS cells. Pharmaceutical agents that modulate cardiac ion channel activity \([21–22,24–28]\), allele-specific RNA interference \([23]\) and \(\beta\)-adrenergic blocking agents \([24,26]\) have been employed to study the LQTS phenotype \(in vitro\). Drug screening of LQTS iPS cells provides a powerful resource to develop individualized clinical regimens for LQTS patients.

In addition to LQTS, patient-specific iPS cell models have also been described for catecholaminergic polymorphic ventricular tachycardia (CPVT) \([29–34]\). While CPVT has two primary genetic causes, mutations in \(R Y R 2\) (CPVT1) or \(C A S Q 2\) (CPVT2), the arrhythmic disease phenotype is directly linked to abnormal calcium handling. Studies of CPVT patient-derived iPS cells have reproduced the arrhythmic signature of the disease in cardiomyocytes \(in vitro\). Specifically, iPS cell-derived cardiomyocytes from CPVT patients display delayed after depolarizations, which are aggravated by catecholaminergic stress and rescued by RYR2 blockers \([32]\), CaMKII inhibitors \([29]\), SERCA inhibitors, antiarrhythmic agents and \(\beta\)-blockers \([30]\). The capacity of CPVT patient-specific iPS cells to model the disease phenotype provides a strong platform with which to develop new drugs or optimize current treatment strategies for this disease.

Patient-specific iPS cell models have also been generated from additional cardiac channelopathies. As with studies of LQTS and CPVT, each cardiac channelopathy modeled by patient-derived iPS cells has focused on beating cardiomyocytes as the cellular phenotype to model disease (Table 1). Importantly, each patient-specific cardiac disease model has demonstrated the capacity to recapitulate cell-autonomous hallmarks of disease. For example, a decrease in sodium current density has been demonstrated in patient-specific iPS cell-derived cardiomyocytes with a sodium channel overlap syndrome \([35]\). iPS cells from patients with Jervell and Lange-Nielson syndrome (JLNS) have documented prolonged action potential duration in differentiated cardiomyocytes compared with healthy controls \([36]\). In iPS cell models of Timothy syndrome, differentiated ventricular-like cardiomyocytes show prolonged action potential duration as well as excess calcium influx and abnormal calcium transients when compared with control cells \([37]\). Overall, iPS cell models of cardiac channelopathies have been successful in recapitulating the disease electrophysiology associated with known genetic defects.

### Table 1. Recent human induced pluripotent stem cell-based models of cardiac channelopathies and cardiomyopathies (cont.)

<table>
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<tr>
<th>Disease classification</th>
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<th>Cell product</th>
<th>Molecular markers</th>
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<td>ACTN2, MYH7</td>
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<td></td>
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<td>Dermal fibroblasts</td>
<td>Beating CMs</td>
<td>ACTN2, TNN13, MYL2, MYH6, MYH7, NKX2.5, HAND1, ANP</td>
<td>[47]</td>
</tr>
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ARVC: Arrhythmogenic right ventricular cardiomyopathy; BTHS: Barth syndrome; CM: Cardiomyocyte; CPVT: Catecholaminergic polymorphic ventricular tachycardia; DCM: Dilated cardiomyopathy; HCM: Hypertrophic cardiomyopathy; JLNS: Jervell and Lange-Nielson syndrome; LQTS: Long QT syndrome.
Interestingly, basal p-ERK levels were increased in LEOPARD iPS cells compared with healthy controls, though additional studies are required to determine if this early difference contributes to the development of hypertrophy in differentiated cardiomyocytes.

Additional cardiomyopathy models have been generated from patient-specific iPS cells with HCM [22,39–40]. Compared with healthy controls, iPS cell-derived cardiomyocytes from HCM patients showed increased cellular size, sarcomeric disorganization and greater nuclear localization of NFATC4, all signatures of a hypertrophic state. These HCM cardiomyocytes were also more sensitive to pharmacologically increased action potential duration and demonstrated a higher propensity for drug-induced arrhythmias [22]. Another study described the aggravating influence of Endothelin 1 on the hypertrophic state of HCM iPS cell-derived cardiomyocytes. While this study saw fewer baseline differences between HCM and healthy control cardiomyocytes, the description of an extracellular factor that can modulate disease phenotype in vitro has advanced the study of HCM disease pathogenesis [40]. In a separate study of familial HCM, cardiomyocyte enlargement and contractile arrhythmia were linked to abnormal calcium dynamics in HCM cardiomyocytes. Specifically, abnormal calcium cycling and increased intracellular calcium levels could be pharmacologically modulated to reduce the hypertrophic characteristics of patient-derived cardiomyocytes in vitro [39]. Collectively, these studies document the capacity of iPS cell-derived cardiomyocytes to recapitulate HCM phenotypes, model clinical susceptibility to drug-induced cardiotoxicity and screen for novel pharmaceutical agents or extracellular factors that modulate disease.

Similar disease models have also been documented by using iPS cells from patients with dilated cardiomyopathy (DCM) [22,41–43]. Studies of iPS cell-derived cardiomyocytes from DCM patients have highlighted disease characteristics such as sarcomeric disorganization, abnormal calcium handling, decreased contractility and increased cellular stress in response to β-adrenergic stimulation [22,41]. In one study, iPS cells were derived from a DCM patient with a novel mutation in DES, an intermediate filament protein involved in cytoskeleton maintenance in cardiomyocytes. This study described abnormal calcium dynamics and impaired response to inotropic stress in patient-derived cardiomyocytes. Importantly, control iPS cells transduced with the mutant DES gene-recapitulated disease phenotypes upon cardiac differentiation. This study highlights the capacity of iPS cells to functionally validate a suspected genetic cause of DCM [42]. Another study documents an increase in nuclear senescence and cellular apoptosis upon stimulating DCM iPS cell-derived cardiomyocytes with a field electrical stress [43]. Overall, cell-autonomous DCM phenotypes can be recapitulated by using patient-specific iPS cells and in vitro differentiation into beating cardiomyocytes.

Recently, iPS cell models of arrhythmogenic right ventricular cardiomyopathy (ARVC) [44,45], Barth syndrome (BTHS) [46], Pompe disease [47] and viral-induced cardiomyopathy [48] have also been described. In vitro models of ARVC have used iPS cell-derived cardiomyocytes from patients with mutations in PKP2. These studies have documented decreased and distorted expression of desmosomes on the cell surface and increased lipid droplet clusters in patient-derived cardiomyocytes versus healthy controls [44,45]. The ability to aggravate the disease phenotype with adipogenic stimuli and mitigate the abnormalities with GSK3β inhibition provides mechanistic insight into early ARVC disease pathogenesis [44]. Patient-specific models of BTHS, a mitochondrial disorder with associated cardiomyopathy, show typical disease characteristics such as abnormal sarcomere assembly, decreased cardiomyocyte contractility and increased levels of reactive oxygen species [46]. Another recent in vitro model of cardiomyopathy used iPS cells derived from patients with Pompe disease [47]. In this study, iPS cells were generated from two patients with mutations in GAA and showed a glycogen storage defect in the pluripotent state. Interestingly, the nuclear reprogramming event was only successful following doxycycline-inducible rescue with a GAA transgene. In this way, autologous control cell lines were generated alongside of patient-specific diseased cell lines (demonstrated to have no transgene integration). Following in vitro differentiation into beating cardiomyocyte-like cells, the authors describe a more pronounced disease phenotype including increased glycogen levels, large glycogen storage vacuoles and abnormal mitochondria. Notably, iPS cell models have also been generated for viral-induced cardiomyopathy [48]. This study investigated the infection of human iPS cell-derived cardiomyocytes with coxsackievirus B3 and demonstrated that this model could effectively predict antiviral drug efficacy and provide mechanistic insights into viral treatment strategies [48]. Collectively, these iPS cell models of diverse cardiomyopathies support the powerful capacity of patient-specific cellular platforms to recapitulate molecular signatures of disease.

In summary of the disease modeling studies reviewed herein, it is generally accepted that iPS cell technology offers a useful patient-specific platform to study cell-autonomous disease phenotypes in vitro. From Table 1, we point out that each patient-specific disease modeling study utilizes beating cardiomyocytes as the cellular phenotype to model disease. However, there is no
consensus between the referenced studies as to what transcriptional or protein markers best characterize an iPS cell-derived cardiomyocyte or definitive markers of maturity. This inconsistency in the field of iPS cell-based cardiac applications is acknowledged in a recent study that proposes a ratio of fetal to adult TNNI isoforms as a uniform measure of cardiomyocyte maturity across independent laboratories [49]. Indeed, the reliability of generating cardiomyocytes of uniform maturation is a current challenge in the field and the caveat remains that iPS cell-derived cardiomyocytes often correspond to a fetal state [50]. While increased consistency in cardiomyocyte characterization would be a significant step forward, we propose that increased emphasis on the timeline of disease development during in vitro differentiation could further accelerate the field. In the following section, we highlight the advantages of disease discovery applications that focus on the process of cardiac differentiation in addition to the cardiomyocyte products.

**Disease discovery: identifying the initial point of disease divergence**

Beyond disease modeling applications, iPS cell technology also promises to advance the discovery of corrupted processes that underlie disease pathogenesis [51]. Harnessing the ability of iPS cells to transition through developmental stages in vitro, we can now shift our focus to the process of proper differentiation in addition to the end product of iPS-derived progeny (Figure 1). The dynamic transition from pluripotent stem cell to increasingly mature somatic tissue encompasses many intermediate progenitor stages. It is at these inflection points in the developmental timeline of iPS cell differentiation that we can now engage in discovery science. For the first time, the developmental roadmap of patient-specific congenital disease phenotypes can be deconvoluted through iPS cell differentiation. This process-oriented focus informs disease pathogenesis by pinpointing the initial divergence between health and disease. By mapping molecular profiles of progressive cellular stages, we can now identify the onset of molecular dysregulation prior to any cellular or organismal phenotype. While this process-focused approach may best serve discovery applications focused on congenital heart disease (CHD), a separate point of divergence may be identified that corresponds to late-onset adult cardiac phenotypes (Figure 2). In this way, iPS cells can inform the development of novel therapeutics that target the initial point of disease pathogenesis, whether in congenital or late-onset disease.

To emphasize the importance of process-focused disease discovery applications of iPS cells, a developmental view of health and disease is critical [51]. Rather than defining health and disease based on comparisons of mature cell phenotypes, we must strive to understand disease as a developmental process with a definable divergence point from health. Figure 2 defines health as the entire developmental timeline between point A (pluripotent stem cell) and point B (healthy mature cell). By contrast, CHD is defined as the entire pathway from point A to point C (diseased mature cell), with a discrete divergence point along the way. Similarly, late-onset adult cardiac disease is defined by the trajectory between point B to point C, with a separate divergence point between the two adult cell phenotypes. It is at these distinct divergence points between the dynamic processes of health and disease that we can identify disease pathogenesis. This temporal approach will enable precise interrogation of cellular dysfunction at early progenitor stages and the development of therapeutic strategies that target the prephenotypic manifestation of disease.

In the case of cardiovascular disease discovery, it is critical to understand the nature of the intermediate progenitor cells between the pluripotent state and the mature cardiomyocyte. A recent study of cardiac progenitors uncovers key transcriptional profiles that inform the staging of these cell types along a developmental timeline [52]. In this study, microarray analysis of murine cardiac-derived progenitor cells determined unique transcriptional signatures across different cell types. Based on the expression levels of known stemness or cardiomyocyte-specific genes, the authors staged the progenitor cells according to their level of cardiac commitment. From earliest (least cardiac committed) to latest (most cardiac committed), the progenitor stages are: ckit+ cells, side population cells and Sca1+ cells [52]. As evidenced by this study, there are defined cellular stages within the healthy mouse heart. Recently, single-cell transcriptional profiles were also described for early embryonic cardiomyocytes and mES cell-derived cardiac progenitor cells and cardiomyocytes [53]. This study further refines our understanding of cardiac lineages within the embryo and those generated from in vitro differentiation. As these studies demonstrate, it is critical to understand the developmental stages of normal cardiogenesis in order to calibrate future disease discovery efforts to a defined timeline. Moreover, a standardized roadmap of normal heart development is critical to inform temporal studies of disease pathogenesis using iPS cells.

**Recent progress in cardiac disease discovery**

Toward the goal of defining the molecular timeline of heart development, recent studies describe a transcriptional atlas of normal cardiogenesis from the pluripotent state to adult cardiomyocytes [54,55]. In these
studies, authors map the dynamic transcriptional landscape of murine heart development from an unbiased genomic perspective. Through time-course transcriptome analysis, they describe the first comprehensive map of gene expression from embryonic stem cells to adult ventricular tissue. These studies also interrogated spatial transcriptional patterns between left ventricle (LV) and right ventricle (RV) development. Interestingly, the authors highlight a greater transcriptional divergence between temporal stages of cardiac development than between distinct LV and RV structures. These data indicate that differences in ventricular tissue may depend on posttranslational modifications or changes induced by mechanical stress. As current cardiac differentiation protocols often generate mixed populations of right and left ventricular cardiomyocytes, insight into subpopulation specification could greatly advance both cardiac disease modeling and disease discovery application of iPS cells [50].

Additionally, the unbiased generation of a transcriptional atlas of cardiac development identified greater complexity in dynamic transcriptional networks during normal cardiogenesis than previously appreciated [54]. From the vast numbers of genes that change during heart development, a disease-centric dynamic interactome has been established based on known genetic variants in CHD [54]. This critical filter enabled identification of stage-specific regulatory networks and hubs that underlie the pathogenesis of CHD. Importantly, these datasets provide a natural context of heart development to better uncover the balance between health and disease. Disease discovery efforts focused on congenital cardiac disease can now be oriented according to an embryo-defined roadmap of cardiogenesis and informed by the dynamic nature of native developmental signatures. By providing a benchmark of normal cardiac development, this transcriptional atlas of cardiogenesis can gauge ongoing studies of cardiac pathogenesis. Overall, these studies provide a developmental context in which to calibrate future in vitro studies of cardiac disease development and progression. Recently, this temporal framework has been harnessed in a study of Rbm20-linked DCM [56]. This disease-discovery investigation utilized the dynamic transcriptional profiles of normal cardiac development to inform a stage-wise, mechanistic model of DCM onset.

In this study [56], authors knocked down Rbm20, an RNA binding protein that functions in cardiac transcript splicing. As mutations in Rbm20 are associated with an early onset cardiomyopathic phenotype, they hypothesized a critical role of Rbm20 in embryonic heart development. Through the use of a high-throughput pluripotent model system and unbiased RNA sequencing analysis, this study identified an early point of transcriptional divergence (D12 of cardiac differentiation) that preceded the cellular phenotype (D24) in mES cell-derived cardiomyocytes. The divergent molecular signature identified differential expression of key cardiac genes including Nkx2.5 and Tnnt2 as well as aberrant splicing of Mef2a and Relb. These findings indicate an initial dysregulation in RNA-processing of cardiac transcription factors and aberrant expression of cardiac gene networks prior to phenotypic onset of disease. Thus, the authors provide evidence that a pluripotent stem cell platform can uncover initial signatures of disease pathogenesis.

Within the same mES cell model of Rbm20-linked DCM, authors discovered differential expression of extracellular matrix (ECM) genes, previously noted in physiological models of DCM but believed to be compensatory in nature. This study suggests that the change in ECM gene expression may be independent of physiological compensation and more directly related to the molecular etiology of Rbm20-linked DCM. Herein, we see the powerful capacity of in vitro disease models to distinguish between cell-autonomous drivers of phenotype and physiological adaptation to disease. This important distinction enables researchers to tease apart molecular mechanisms of disease pathogenesis in the absence of confounding physiological compensation. Overall, this study highlights the developmental nature of Rbm20-linked DCM as a disease that is patterned during early cardiogenesis and progressively unravels due to pathogenic cardiac remodeling. Additional
studies of this nature are required to accelerate our understanding of progenitor cell contribution to disease and inform the development of novel therapeutic strategies that target earlier stages of disease.

**Challenges in iPS cell disease modeling & disease discovery**

While significant progress is continuing to be made using iPS cells as a platform for cardiac disease modeling and disease discovery, the field remains limited by challenges in both the reprogramming and differentiation phases of these experiments [50]. Future advances in disease modeling and disease discovery studies will require greater predictability and consistency in cardiac differentiation of individual iPS cell lines. Moreover, increased specificity in cardiomyocyte subtype differentiation and the generation of mature cardiomyocyte phenotypes will be necessary to unravel precise mechanisms of late-onset disease pathogenesis. In addition, improvements in the scalability of *in vitro* cardiac differentiation will accelerate high-throughput drug-screening applications of iPS cell-derived cardiomyocytes.

Differences in nuclear reprogramming strategy, including the identity of pluripotency transgenes and the integrating or nonintegrating nature of transduction, can significantly variegate iPS cell cardiogenicity [57–59]. Moreover, residual epigenetic signatures of the starting material may introduce differentiation bias toward a cellular phenotype more closely related to the somatic origin [60–62]. Further complexity is added by the clonal variability in cardiogenic capacity [59] that exists across iPS cell lines from an individual patient even when reprogramming strategy and somatic origin are held constant. Additional research must be conducted to determine the optimal somatic origin and reprogramming strategy to yield consistent and reliable differentiation output from iPS cells.

The heterogeneous nature of iPS cell-derived cardiomyocytes is also a significant limitation of current cardiac disease modeling and disease discovery applications. Variability in the percentage of cardiac tissue within an iPS cell-derived population [50,59] reveals the current challenge of efficient and reliable cardiac differentiation. In addition, within the cardiac population of cells, there is often an unpredictable mix of cardiomyocyte subtypes: atrial, ventricular and nodal [50]. Recent work has been conducted to modulate cellular signaling during cardiac differentiation and regulate the ratio of resulting atrial/ventricular to nodal cardiomyocytes [63]. Additional studies will be necessary for researchers to gain the precise control needed to study specific cardiomyocyte subtypes *in vitro*.

Insights gained from iPS cell models of disease are also affected by the limited maturation of iPS cell-derived cardiomyocytes. Most often, *in vitro* cardiomyocytes only achieve a fetal phenotype in contractile machinery and electrophysiological properties [50]. While recent work has highlighted factors such as mechanical force that can induce sarcomeric maturity within iPS cell-derived cardiomyocytes [64], much more research is needed to consistently generate adult-like cardiomyocytes *in vitro*. To advance process-focused disease discovery applications, additional studies will be required to determine the accuracy with which iPS cell-derived cardiac progenitors align with natural progenitor stages during *in utero* heart development [55].

Furthermore, advances in drug-screening applications of iPS cell-derived cardiomyocytes will require the development of differentiation protocols that are scalable to high-throughput production. Recent advances in suspension culture of iPS cells enable expansion of high-quality undifferentiated cells beyond the limits of traditional adherent cell culture [65]. In addition, progress in electrophysiological assays using multielectrode arrays now enables reliable and high-throughput readouts for *in vitro* drug screening using iPS cell-derived cardiomyocytes [66]. Overall, while recent and ongoing studies have greatly advanced the specificity and reproducibility of iPS cell-based cardiac differentiation, much work remains to be done to optimize the generation of *in vitro* cardiomyocytes for disease modeling and disease discovery applications.

**Conclusion**

In conclusion, iPS cell-based disease modeling and disease discovery applications continue to inform our understanding of a variety of cardiovascular diseases. The utility of these model systems will be enhanced through advances in the reproducibility of nuclear reprogramming and *in vitro* cardiac differentiation, the predictable generation and purification of iPS cell-derived cardiac phenotypes, as well as the production of fully mature iPS cell-derived cardiomyocytes. Through complementary product-focused disease modeling and process-focused disease discovery applications, iPS cell-based platforms will continue to revolutionize the field of cardiovascular biology.

**Future perspective**

As the field of bioengineered stem cells continues to advance, there is particular excitement for increased focus on the process of *in vitro* differentiation to complement studies of the final cell phenotype. In this way, the field can harness the full potential of iPS cells to uncover the initial pathogenesis of car-
Induced pluripotent stem cells for cardiovascular disease. The individualized nature of this disease discovery platform enables for the first time visualization of patient-specific, cell-autonomous disease development. This evolution from product-focused disease modeling to further encompass process-focused disease discovery brings the opportunity to better understand prephenotypic disease onset and lays the groundwork for targeted individualized therapeutics. We can thereby envision a transformation of clinical practice, wherein preventative in utero therapeutics will target causative molecular defects that underlie disease pathogenesis.

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Executive summary
Product-focused disease modeling
- Various cardiac channelopathies and cardiomyopathies have been successfully modeled in vitro using beating induced pluripotent stem (iPS) cell-derived cardiomyocytes from diseased patients.
- Patient-specific iPS cell platforms are currently being utilized to study the cell-autonomous nature of disease and recapitulate disease phenotypes at the cellular level.
- Direct comparison between iPS cell-derived cardiomyocytes from patients and controls can identify an individualized signature of disease.

Process-focused disease discovery
- The process of in vitro differentiation can be studied to pinpoint the initial molecular divergence between health and disease.
- A transcriptional atlas of normal cardiogenesis can inform temporal studies of cardiac disease pathogenesis.
- Identification of the initial point of disease pathogenesis will enable novel therapeutic strategies that target the earliest molecular signature of disease.

References


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