The prospect of changing the plasticity of terminally differentiated cells toward pluripotency has completely altered the outlook for biomedical research. Human-induced pluripotent stem cells (iPSCs) provide a new source of therapeutic cells free from the ethical issues or immune barriers of human embryonic stem cells. iPSCs also confer considerable advantages over conventional methods of studying human diseases. Since its advent, iPSC technology has expanded with 3 major applications: disease modeling, regenerative therapy, and drug discovery. Here we discuss, in a comprehensive manner, the recent advances in iPSC technology in relation to basic, clinical, and population health.

Since the initial discovery that bone marrow cells possess regenerative capacity through clonal expansion, which laid the foundation for the field (1), regenerative medicine has come a long way. As a type of stem cell, these bone marrow cells have the capacity for both self-renewal and differentiation into different cell types. Earlier attempts to understand the pluripotency of the inner cell mass focused on the developmental capacity of nuclei by cloning in frogs (2), cloning in adult mammalian cells (3), derivation of mouse and human embryonic stem cells (ESCs) (4,5), and generation of ESCs and somatic cell fusion (6). Similarly, MyoD, a mammalian transcription factor, was found capable of converting fibroblasts to myocytes, which led to the concept of master regulators, transcription factors that determine lineage specification (7). Using these paradigms, subsequently discovered stem cells were classified as either adult stem cells or ESCs based on their origins and differentiation potentials. The unusual capacities of ESCs to proliferate without senescing (i.e., self-renewal) and to form all cell types of the embryo (i.e., pluripotency) made them unique and valuable resources for studying cell fate and tissue development. Initially, work on pluripotent stem cells (PSCs) was conducted using human ESCs (5); however, the requirement to destroy early-stage embryos in the process of ESC derivation made their use ethically controversial. In addition, practical considerations hindered their medical applications, because any cells or tissues generated from human ESCs by definition would be allotransplants into the recipient patient, requiring possible life-long immunosuppression therapy.

The discovery by Takahashi et al. (8,9) that a small set of reprogramming factors (e.g., Oct4, Sox2, Klf4, ...
and c-myc; OSKM) can induce nuclear reprogramming of mouse (8) and adult human cells (9) to pluripotency became a landmark development in regenerative medicine. Termed induced pluripotent stem cells (iPSCs), they promised a source of therapeutic cells free from the ethical issues or immune barriers of human ESCs while retaining similar properties, such as self-renewal and pluripotency. Since that initial discovery, a number of advances have been introduced to enhance both the efficiency of iPSC production and the safety of the resultant lines. These improvements include the use of chemical agents to enhance efficiency (BIX-01294, valproic acid, RG108, AZA [5-aza-2’-deoxycytidine], dexamethasone, TSA [trichostatin A], and A-83-01) (10,11), use of alternative cell sources for reprogramming (embryonic, fetal, and adult fibroblasts; neural stem cells; adipose stem cells; keratinocytes; and blood cells) (12-14), use of various factors that could replace the reprogramming factors (15,16), use of vectors that can be excised from the genome (17,18), use of “nonintegrating” vectors (19,20), supplementation of reprogramming factors with microribonucleic acids (21), use of recombinant proteins/peptides to reprogram somatic cells (22,23), and most recently, a purely chemical approach (24).

With the concerted efforts of the academic community, the past decade has seen a tremendous push toward the efficient generation of safer PSCs, with the hope that one day iPSCs could be used for regenerative medicine in the clinic (25). Although halted temporarily, the clinical trial using iPSC-derived retinal pigment epithelial cells (iPSC-RPEs) for treatment of macular degeneration shows how far we have come in developing clinical-grade stem cells (26). In the short run, iPSC technology will likely offer more avenues to understand the pathophysiology of diseases and discover new therapeutic molecules. Specifically, for disease modeling, iPSCs can be generated from patients who carry certain genetic mutations and then differentiated into disease-relevant cell types, such as cardiomyocytes (iPSC-CMs) (27-29). Similarly, iPSC-derived cells can then be subjected to high-throughput screens to discover new therapeutic small molecules or conduct drug toxicity assays. Lastly, iPSC technology might enable personalized therapies (i.e., cell or tissue replacement without the need for immunosuppression, and use of drugs tailored according to each patient’s genes, environment, and life-style). This is precision medicine, an initiative with the intent to cure each patient by taking into account his or her unique genetic makeup (30). The goal is to understand the complex mechanism of diseases so that made-to-order treatment plans could be designed for each patient based on their condition. In this review paper, we aim to highlight the promise of iPSCs for expanding basic science research and generating novel therapeutic agents for clinical and public health applications.

**IPSC TECHNOLOGY: EMERGING CONCEPTS**

The greatest hallmark of iPSC technology lies in its simplicity and reproducibility. Although the past decade has shown great improvement in making iPSCs safer and more efficacious, which is critical for pushing the technology toward clinical application, the mechanisms involved in efficient generation of iPSCs are just emerging. These evolving concepts raise important fundamental questions that will be discussed in the following sections.

**HOW CAN A FEW TRANSCRIPTION FACTORS TURN BACK THE CELLULAR CLOCK?** Many studies have tried to address this question, but the general consensus is that the initial activity of the core pluripotency genes (OSKM) has a snowball effect that results in simultaneous activation of the entire endogenous network of pluripotency genes and inhibition of lineage-specific genes within the reprogrammed somatic cells. The initial phase of reprogramming is associated with cells undergoing metabolic changes and genome-wide alterations in histone marks and methylation, followed by a late maturation phase that causes defined changes in nuclear structure, the cytoskeleton, and signaling pathways (31,32). Indeed, by looking closely at these mechanisms, researchers can now obtain nearly perfect iPSCs by clearing previous roadblocks to reprogramming (33).

**ARE THESE iPSCs THE SAME AS ESCs?** An important question that comes up repeatedly is whether iPSCs have the same genetic and epigenetic landscape as ESCs, and whether differentiated cells (e.g., CMs) from these 2 types of stem cells behave similarly. Despite several previous studies suggesting differences in gene expression or deoxyribonucleic acid (DNA) methylation between iPSCs and ESCs (34–36), the majority of iPSC and ESC clones are largely indistinguishable (37–39). More recent reports suggest that both cell types have identical molecular and functional characteristics, with variations in their genetic backgrounds (i.e., different donors for iPSCs and ESCs) accounting for most of their regulatory differences (40).
COULD TRANSDIFFERENTIATION BE THE NEXT STEP? Another emerging concept is the direct reprogramming of one somatic cell type to another desired cell type as an alternative approach to iPSCs. Termed transdifferentiation, this concept relies on the premise that we could achieve rapid reprogramming of a desired cell type by entirely avoiding the iPSC stage (41). Several groups have shown successful transdifferentiation of fibroblasts to various types of somatic cells, such as neurons (42), hepatocytes (43), CMs (44), and endothelial cells (45,46). However, a current major drawback to the use of direct reprogramming for clinical purposes is the tremendous difficulty of obtaining sufficient numbers of target cells that fully recapitulate the properties of the desired cells (47). In addition, transdifferentiation has thus far failed to generate a pure population of desired cells, which makes it difficult for them to be used for disease modeling and drug development (48). Similarly, in contrast to iPSCs, which once reprogrammed can be easily maintained and differentiated to a desired cell type, the transdifferentiation approach would require restarting the entire reprogramming process for each experiment.

APPLICATIONS OF iPSCs

There are many human diseases that have limited treatment options because of either a lack of relevant tissue samples or a lack of information regarding disease progression. Thus, researchers traditionally have relied on in vitro assays or animal models to understand disease progression and develop therapeutic interventions. These model systems use either small animals (e.g., rats and mice) or large animals (e.g., dogs, pigs, and nonhuman primates). These research designs are premised on the ability of the animal model to mimic human subjects pathophysiologically and to eventually develop end-stage disease like that seen in humans. However, because of differences in cardiovascular anatomy and physiology (e.g., humans and rodents diverged ~75 million years ago), animal models often do not accurately reproduce human pathophysiology in meaningful ways. Because iPSCs can be derived from healthy and diseased patients, they can be a robust alternative to animals for modeling human diseases (Figure 1). Indeed, several models of iPSCs have been generated and explored for studying various human diseases, as outlined in the next section.

DISEASE MODELING. The inherent properties of iPSCs for self-renewal and differentiation into any cell type make them an ideal candidate for modeling human diseases. Because many of the genetic variants that distinguish affected patients from unaffected subjects are located in noncoding regions of the human genome with limited alignment to genomes of animals used as model systems, we run the risk that even after introducing the same human genetic variant in the animal model, we might fail to recapitulate the human disease phenotype (49). Thus, there is a compelling need to model human diseases using human samples. Since the groundbreaking creation of the first human iPSCs (9), many groups have applied this technology to model human diseases, with amyotrophic lateral sclerosis and various congenital diseases among the first to be modeled using patient-specific iPSCs (12,50,51). Since these reports, many have attempted to study human diseases using iPSCs from patients with neurodegenerative diseases (50,52), cardiovascular disorders (53-56), muscular dystrophies (57,58), and hematologic disorders (59,60), to name a few. Cardiovascular diseases that have been studied are summarized in Table 1.

Modeling a broken heart: use of iPSCs. Cardiomyopathy is a complex disease of the heart that has a pathogenesis that includes myocardial infarction, genetic mutations, endocrine disease, and drug toxicities. Historically, animal models have been used to understand the pathophysiology of cardiovascular diseases and discover new therapeutics; however, because of significant differences in cardiovascular genetics and physiology between humans and animals, there is a compelling need for more accurate models to understand cardiac diseases. The ability to differentiate iPSCs to disease-relevant derivatives, such as iPSC-CMs (27,77), now offers a valuable opportunity to derive patient-specific cells for the purpose of cardiac disease modeling and drug discovery (28,78).

Human iPSC-CMs can serve as a disease model in a dish by retaining the genetic variance present in the patient and eventually exhibiting the phenotypic features of the disease in vitro. Indeed, several groups have used this iPSC-CM technology to model cardiac channelopathies, such as long-QT syndromes (53-55,71,79,80), Timothy syndrome (73), LEOPARD syndrome (51), catecholaminergic polymorphic ventricular tachycardia (81,82), arrhythmogenic right ventricular dysplasia (74,83), familial hypertrophic cardiomyopathy (62), and familial dilated cardiomyopathy (64-66). In addition to serving as an aid in the understanding of genetic cardiomyopathies, iPSC-CMs have been used to model acquired or extrinsic cardiac diseases caused by endocrine, metabolic, and neuromuscular dysfunction. For example, iPSC-CMs are being used in cell-based assays to model
susceptibility to drug-induced cardiotoxicity (84,85), thereby allowing researchers to predict adverse drug responses more accurately in patients with different genetic backgrounds. Similarly, iPSC-CMs have been used to understand the disease progression of diabetic cardiomyopathy (86), viral myocarditis (87), cardiac hypertrophy (88), and genetic polymorphism-induced cardiac ischemia (89).

Disease modeling using iPSC-CMs has been feasible because of ever-improving differentiation protocols (27,29,90). Importantly, although the phenotypic characteristics of the iPSC-CMs are similar to those of primary human CMs with respect to their molecular, electrophysiological, mechanical, and metabolic properties, these functional characteristics also indicate that they resemble fetal rather than adult CMs. Structurally, compared with adult human CMs, these iPSC-CMs look much smaller in size and have less sarcomeric organization with decreased force generation (91–93). This could diminish their utility for modeling adult-related heart diseases. Table 2 summarizes the differences between immature and mature CMs.

**Table 2**

<table>
<thead>
<tr>
<th>Immature CMs</th>
<th>Mature CMs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smaller in size</td>
<td>Larger in size</td>
</tr>
<tr>
<td>Less sarcomeric organization</td>
<td>More sarcomeric organization</td>
</tr>
</tbody>
</table>

**iPSC-CMs need to mature.** In the developing heart, networks of diverse factors tightly regulate cardiomyocyte maturation, including mechanical, electrical, and biochemical signals. Although relatively simple expedients, such as keeping iPSC-CMs in culture for prolonged periods (97) or growing them on specific substrates (119), can enhance some aspects of
their structure and function, much research now focuses on uncovering factors and pathways that drive their maturation (120). Agents and cues that have been explored to induce maturation include microRNAs (121,122), chromatin and histone proteins (123), DNA methylation (124), metabolic energetics (125), and biochemical cues (126,127). By decoding these signaling pathways, substantial progress has been made in generating more mature iPSC-CMs. Additional future studies using high-throughput experiments would be necessary to unravel the complexity of full cardiac maturation. Unraveling these pathways could potentially allow us to bring iPSC-CMs to maturity in a dish, with closer resemblance to the adult human heart. However, it is unlikely that cells grown under in vitro conditions will fully resemble cells residing in intact living subjects because of differences in environmental milieu, whether cardiac or noncardiac. Hence, the expectation that iPSC-CMs will be identical to mature primary human CMs might be unrealistic.

**iPSCs and genome engineering: a perfect match.** The emergence of genome editing tools, such as zinc finger nucleases, transcription activator-like effector nucleases, and the clustered regularly interspaced short palindromic repeat (128), has further advanced the application of iPSC technology to precision medicine. These genetic engineering techniques allow for DNA to be inserted, replaced, or removed from a genome by use of nucleases that create specific double-strand breaks at preferred locations. Endogenous cellular mechanisms then repair these breaks by homologous recombination (129). With these tools, it is now possible to generate human cellular disease models in a precise and predictable manner. Indeed, genome editing has been used to introduce genetic alterations to create cardiac disease models or correct genetic mutations in iPSC-CMs to model cardiac diseases. Single genetic mutations responsible for cardiomyopathies, such as dilated cardiomyopathy, Barth syndrome, long-QT syndrome, and Duchenne muscular dystrophy, have been corrected by use of these genome-editing tools (68,70,71,130,131), which suggests the feasibility of this approach for disease modeling. However, these engineered nucleases could introduce unintended genomic alterations; for example, in addition to cleaving the on-target site, they might have off-target effects. Similarly, other challenges, such as delivery methods and efficiency, still need to be sorted out (132). Taken together, this tag-team technology of patient-specific iPSCs and genome editing could lay the groundwork required to achieve the bold initiative of precision health (30).

### TABLE 1 List of Cardiac Diseases Modeled With iPSCs

<table>
<thead>
<tr>
<th>Disease</th>
<th>Locus</th>
<th>Features</th>
<th>Gene Correction</th>
<th>Gene Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPVT</td>
<td>CASQ2</td>
<td>Arrhythmia and abnormal Ca&lt;sup&gt;2+&lt;/sup&gt; signaling</td>
<td>No</td>
<td>(61)</td>
</tr>
<tr>
<td></td>
<td>RYR2</td>
<td>Arrhythmia and abnormal Ca&lt;sup&gt;2+&lt;/sup&gt; signaling</td>
<td>No</td>
<td>(61)</td>
</tr>
<tr>
<td>HCM</td>
<td>MYH7</td>
<td>Abnormal Ca&lt;sup&gt;2+&lt;/sup&gt; handling, disorganized sarcomeres, and electrophysiological irregularities</td>
<td>No</td>
<td>(62)</td>
</tr>
<tr>
<td>DCM</td>
<td>LMNA</td>
<td>Nuclear senescence and cellular apoptosis</td>
<td>No</td>
<td>(63)</td>
</tr>
<tr>
<td></td>
<td>TNNT2</td>
<td>Altered regulation of Ca&lt;sup&gt;2+&lt;/sup&gt;, decreased contractility, and abnormal distribution of sarcomorphic z-actin</td>
<td>No (64,65)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TTNTv</td>
<td>Sarcomere abnormalities, impaired contractility and response to stress</td>
<td>No</td>
<td>(66)</td>
</tr>
<tr>
<td></td>
<td>DES</td>
<td>Abnormal desmin aggregations, aberrant Ca&lt;sup&gt;2+&lt;/sup&gt; handling, and beating rate</td>
<td>No</td>
<td>(67)</td>
</tr>
<tr>
<td></td>
<td>PLN</td>
<td>Abnormal Ca&lt;sup&gt;2+&lt;/sup&gt; handling, electrical instabilities, and increased cardiac hypertrophy markers</td>
<td>Yes</td>
<td>(68)</td>
</tr>
<tr>
<td></td>
<td>MYBPC3</td>
<td>Contraction defects</td>
<td>No (69)</td>
<td></td>
</tr>
<tr>
<td>BTHS</td>
<td>TAZ</td>
<td>Sarcomere assembly and myocardiac contraction abnormalities</td>
<td>No</td>
<td>(70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LQT1</td>
<td>KCNJ1</td>
<td>Prolonged duration of AP, aberrant electrophysiological K&lt;sup&gt;-&lt;/sup&gt; current, and protective action of beta-blockade</td>
<td>No</td>
<td>(71)</td>
</tr>
<tr>
<td>LQT2</td>
<td>KCNH2</td>
<td>Prolonged AP duration, reduced cardiac potassium current I (K&lt;sup&gt;-&lt;/sup&gt;), and increased arrhythmogenicity</td>
<td>Yes (54,55,71)</td>
<td></td>
</tr>
<tr>
<td>LQT3</td>
<td>SCN5A</td>
<td>Prolonged AP duration and persistent Na&lt;sup&gt;+&lt;/sup&gt; current</td>
<td>No</td>
<td>(72)</td>
</tr>
<tr>
<td>LQT8</td>
<td>CACN1C</td>
<td>Defects in Ca&lt;sup&gt;2+&lt;/sup&gt; signaling and arrhythmia</td>
<td>No</td>
<td>(73)</td>
</tr>
<tr>
<td>ARVD</td>
<td>PKP2</td>
<td>Exaggerated lipopogenesis, desmosomal distortion</td>
<td>No</td>
<td>(74)</td>
</tr>
<tr>
<td>Pompe disease</td>
<td>GAA</td>
<td>Glycogen accumulation and abnormal ultrastructure</td>
<td>Yes</td>
<td>(75)</td>
</tr>
<tr>
<td>FRDA</td>
<td>FXN</td>
<td>Impaired iron homeostasis, mitochondrial damages and aberrant Ca&lt;sup&gt;2+&lt;/sup&gt; signaling</td>
<td>No</td>
<td>(76)</td>
</tr>
<tr>
<td>LEOPARD syndrome</td>
<td>PTPN11</td>
<td>Abnormal cell size and sarcomere organization</td>
<td>No</td>
<td>(51)</td>
</tr>
</tbody>
</table>

AP = action potential; ARVD = arrhythmogenic right ventricular dysplasia; BTHS = Barth syndrome; Ca<sup>2+</sup> = calcium; CPVT = catecholaminergic polymorphic ventricular tachycardia; DCM = dilated cardiomyopathy; FRDA = Friedreich ataxia; HCM = hypertrophic cardiomyopathy; iPSCs = induced pluripotent stem cells; K<sup>-</sup> = potassium; LEOPARD = lentigines, electrocardiographic conduction defects, ocular hypertelorism, pulmonary stenosis, abnormalities of the genitals, retarded growth, and deafness or hearing loss; LQT = long-QT syndrome; Na<sup>+</sup> = sodium.

**REGENERATIVE MEDICINE.** The use of PSCs, such as ESCs and iPSCs, for cell transplantation or organogenesis has captured the imagination of the lay public, but it remains a difficult task to achieve from a scientific standpoint. The limitations of organ transplantation, which is plagued by lack of organ availability and problems with immunorejection, have led
### Table 2: Molecular, Electrophysiological, and Metabolic Profile of iPSC-CMs and Mature Cardiomyocytes

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Immature CMs</th>
<th>Mature CMs</th>
<th>Ref. #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td>Round or polygonal</td>
<td>Rod and elongated</td>
<td>(94)</td>
</tr>
<tr>
<td>Size</td>
<td>20-30 pF</td>
<td>150 pF</td>
<td>(95)</td>
</tr>
<tr>
<td>Nuclei per cell</td>
<td>Mononucleated</td>
<td>~25% Multinucleated</td>
<td>(96)</td>
</tr>
<tr>
<td>Multicellular organization</td>
<td>Disorganized</td>
<td>Polarized</td>
<td>(95)</td>
</tr>
<tr>
<td>Sarcomere appearance</td>
<td>Disorganized</td>
<td>Organized</td>
<td>(95)</td>
</tr>
<tr>
<td>Sarcomere length</td>
<td>Shorter (~1.6 μm)</td>
<td>Longer (~2.2 μm)</td>
<td>(97)</td>
</tr>
<tr>
<td>Sarcomeric proteins</td>
<td>MHC β &gt; α</td>
<td>β &gt;&gt; α</td>
<td>(98)</td>
</tr>
<tr>
<td>Titin</td>
<td>N2BA</td>
<td>N2B</td>
<td>(99)</td>
</tr>
<tr>
<td>Troponin I</td>
<td>sSnl</td>
<td>cTnI</td>
<td>(100)</td>
</tr>
<tr>
<td>Sarcomere units</td>
<td>Z-discs and I-bands</td>
<td>Formed</td>
<td>(95)</td>
</tr>
<tr>
<td></td>
<td>H-zones and A-bands</td>
<td>Formed (prolonged</td>
<td>(95)</td>
</tr>
<tr>
<td></td>
<td>M-bands and T-tubules</td>
<td>Absent</td>
<td>(95)</td>
</tr>
<tr>
<td><strong>Electrophysiology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Action potential properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting membrane potential</td>
<td>~60 mV</td>
<td>~90 mV</td>
<td>(93)</td>
</tr>
<tr>
<td>Upstroke velocity</td>
<td>~50 V/s</td>
<td>~250 V/s</td>
<td>(101)</td>
</tr>
<tr>
<td>Amplitude</td>
<td>Small</td>
<td>Large</td>
<td>(95)</td>
</tr>
<tr>
<td>Spontaneous automaticity</td>
<td>Exhibited</td>
<td>Absent</td>
<td>(95)</td>
</tr>
<tr>
<td>Ion currents</td>
<td>Hyperpolarization-activated</td>
<td>Present</td>
<td>(101)</td>
</tr>
<tr>
<td></td>
<td>pacemaker (Ih)</td>
<td>Absent</td>
<td></td>
</tr>
<tr>
<td>Sodium (hNa)</td>
<td>Low</td>
<td>High</td>
<td>(102)</td>
</tr>
<tr>
<td>Inward rectifier potassium (hK1)</td>
<td>Low or absent</td>
<td>High</td>
<td>(101)</td>
</tr>
<tr>
<td>Transient outward potassium current (hKo)</td>
<td>Inactivated</td>
<td>Activated</td>
<td>(103)</td>
</tr>
<tr>
<td>ATP-sensitive K+ current (I_{K,ATP})</td>
<td>Not reported</td>
<td>Present</td>
<td>(104)</td>
</tr>
<tr>
<td>L- and T-type calcium (hCa,L and hCa,T), rapid and slow rectifier potassium currents (hCa,L and hCa,T), Na+-Ca²⁺ exchange current (hCa,L) and acetylcholine-activated K⁺ (hK,ACh)</td>
<td>Similar to adult CMs</td>
<td></td>
<td>(93)</td>
</tr>
<tr>
<td>Conduction velocity</td>
<td>Propagation of signal</td>
<td>Slower (~0.1 m/s)</td>
<td>(105)</td>
</tr>
<tr>
<td>Gap junctions</td>
<td>Distribution</td>
<td>Circumferential</td>
<td>(106)</td>
</tr>
<tr>
<td>Calcium handing</td>
<td>Ca²⁺ transient</td>
<td>Inefficient</td>
<td>(107)</td>
</tr>
<tr>
<td></td>
<td>Amplitudes of Ca²⁺ transient</td>
<td>Small and decrease with pacing</td>
<td>(108)</td>
</tr>
<tr>
<td></td>
<td>Excitation-contraction coupling</td>
<td>Slow</td>
<td>(109)</td>
</tr>
<tr>
<td></td>
<td>Contractile force</td>
<td>~nN range/cell</td>
<td>(110)</td>
</tr>
<tr>
<td></td>
<td>Ca²⁺-handling proteins</td>
<td>Positive</td>
<td>(111)</td>
</tr>
<tr>
<td></td>
<td>CASQ2, RyR2, and PLN</td>
<td>Low or absent</td>
<td>(112)</td>
</tr>
<tr>
<td>Force-frequency relationship</td>
<td>Low or absent</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>Mitochondrial bioenergetics</td>
<td>Mitochondrial number</td>
<td>Low</td>
<td>(113)</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial volume</td>
<td>Low</td>
<td>(113)</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial structure</td>
<td>Irregular distribution, perinuclear</td>
<td>(114)</td>
</tr>
<tr>
<td></td>
<td>Mitochondrial proteins</td>
<td>DRP-1 and OPA1</td>
<td>(115)</td>
</tr>
<tr>
<td></td>
<td>Metabolic substrate</td>
<td>Glycolysis (glucose)</td>
<td>Oxidative (fatty acid)</td>
</tr>
<tr>
<td>Responses to β-adrenergic stimulus</td>
<td>Response</td>
<td>Lack of inotropic reaction</td>
<td>(117)</td>
</tr>
<tr>
<td></td>
<td>Cardiac alpha-adrenergic</td>
<td>Absent</td>
<td>(118)</td>
</tr>
<tr>
<td></td>
<td>receptor ADRA1A</td>
<td>Present</td>
<td></td>
</tr>
</tbody>
</table>

ATP = adenosine triphosphate; CM = cardiomyocyte; iPSCs = induced pluripotent stem cells; MHC = major histocompatibility complex.
researchers to seek alternative approaches (9,15). Both ESCs and iPSCs could differentiate into any cell type in the body and thus could potentially replace diseased or dysfunctional cells. Advances in culturing technology and differentiation protocols have produced much safer and clinically relevant cells with minimal tumorigenic risk (25). This enabled several groups to initiate clinical trials using PSCs, with most targeting eye diseases (26). Of the 13 clinical trials being conducted, 9 are for ESC-RPE or iPSC-RPE cells for macular degeneration, which causes the progressive deterioration of light-sensing photoreceptors in the eye (133). Previous work had shown that iPSC-RPE and ESC-RPE cells were safe and functional in pre-clinical models of macular degeneration (134,135). Similarly, iPSCs generated from patients with retinitis pigmentosa, a condition that involves retinal degeneration, were able to differentiate to rod photoreceptor cells and have beneficial effects (136,137). Despite the excitement and momentum for the use of iPSC derivatives in clinical trials, a cautious approach is advisable, because one of the clinical trials focused on treating macular degeneration with iPSC-RPE cells was recently halted because of genetic mutations in the derived autologous cells. In addition, pre-clinical data related to iPSCs have shown great promise for various other ailments, including hematopoietic disorders, spinal cord and musculoskeletal injury, and liver damage (138–141). Because a comprehensive detailing of all these is beyond the scope of this review, we discuss only a few applications to cardiovascular diseases in the following sections.

**Patching the broken heart.** Cardiovascular diseases remain the leading cause of mortality and morbidity in humans. PSC-derived cardiac tissue might help restore diseased areas of the heart. Indeed, early work showed that PSC-CMs not only formed myocardial grafts but also electrically coupled and partially remuscularized the infarcted areas and improved myocardial performance in small animal models (142–145). Similarly, PSC-CMs have shown promise in some pre-clinical large animal models (146). In a recent study, Chong et al. (147) reported remuscularization of the infarcted region when human ESC-CMs were injected into nonhuman primate models of myocardial infarction. These grafts showed synchrony with the host myocardium, with regular calcium transients and electrical coupling after transplantation; however, ventricular arrhythmias were noted in these animals after transplantation, and the study failed to show an improvement in their cardiac function. Clearly, significant hurdles still need to be overcome to translate this pre-clinical report to a clinical trial. A much larger cohort of animals would be required to reach statistical power and allow more in-depth mechanical analyses of the engrafted heart, and the ventricular arrhythmias noted in these animals after transplantation must be addressed before human trials can be conducted (148,149). In an effort to overcome the issues of poor engraftment or survival of transplanted cells, another study showed that a cytokine-loaded 3-dimensional fibrin patch with iPSC-derived cardiovascular cells (iPSC-CMs, iPSC-derived endothelial cells [ECs], and iPSC-derived smooth muscle cells) had a better chance of improving cardiac function in a pig model of myocardial infarction (146). One current clinical trial is actively recruiting patients to test the use of human ESC-derived cardiac progenitor cells in patients with heart failure (150). On the basis of the pre-clinical data showing a significant improvement in cardiac function caused by the paracrine effects of the transplanted cardiac progenitor cells (151), this ongoing clinical trial is using fibrin patches that contain Isl-1+ SSEA-1+ cells. The first clinical case report from this trial has found improvement in the patient’s functional outcomes and evidence of new-onset contractility of the transplanted area (152); however, the evaluation of efficacy remains speculative, because these ESC-fibrin patches were implanted at the time of a coronary artery bypass graft procedure. Accordingly, more patients need to be recruited, and randomized trials need to be performed in the future.

**Tissue engineering: to the rescue of cell therapy.** It has become quite clear in the past few years that without optimal bioengineering tools, the use of PSC-CMs for regenerative medicine might be limited. This is evident given that previous studies using PSC-CMs or progenitor cells have seen only moderate success because of the failure of cells either to survive transplantation or to fully engraft into the host myocardium, which limits possible gains in cardiac function (153). Similarly, misalignment of the engrafted cardiac cells with the host myocardium increases the risk of ventricular arrhythmias caused by electrical heterogeneity (147,148,154). A patch-based approach for cardiac repair might be more advantageous than intramyocardial injections, because the patch could simultaneously act as a substrate to strengthen the injured myocardium and prevent adverse remodeling, as well as provide a template for cells to survive and even proliferate. With advances in tissue bioengineering, diverse biomaterials have been found with the potential to enhance cardiac cell therapy (155). These biomaterials (including collagen, fibrin, alginites, silk, decellularized heart matrix, and synthetic polymers) now allow researchers to deliver
cells, retain them in situ, and use them to replace scar tissue with a large number of stem cell derivatives. In addition to being a vehicle for cell delivery, cardiac patches have been shown to stimulate endogenous paracrine factors that assist in cardiac repair (156,157).

Although there is much enthusiasm for the use of engineered heart tissue constructed from PSC-CMs for cardiac repair, there are some limitations that need to be addressed, including how to engineer tissues that are simultaneously large enough to provide benefits but small enough to prevent death of the transplanted cells because of lack of oxygen and nutrients. Even transplanted engineered heart muscle constructs showed poor survivability because of lack of vascularization (158). Hence pre-vascularization of the in vitro heart tissue could potentially make these engineered patches more closely resemble the native human myocardium and allow survival of thicker tissues. Indeed, the addition of human ECs to ESC-derived human myocardium and smooth muscle cells to a porcine model of myocardial infarction resulted in significant improvements in cardiac function (159). Taken together, the continuing progress made in tissue engineering to promote cell engraftment and survival after transplantation is expected to take us yet another step closer to the clinical use of PSC-CMs in regenerative medicine.

**DRUG DISCOVERY AND TOXICITY SCREENING.** In the past, immortalized cell lines or experimental animal models have been used to screen therapeutic agents for human diseases with limited success because of differences from the actual human setting. For example, it has always been difficult to analyze and compare data between mice and humans for cardiac diseases given the considerable variation in their heart rates (e.g., mice exhibit a higher heart rate of 500 to 600 beats/min compared with human heart rates of 60 to 80 beats/min) and electrophysiological properties (e.g., mice have shorter action potentials than humans because of differences in cardiac ion channels) (160). Studies using a patient’s primary heart tissue are helpful but are limited because of lack of donor availability, which makes in vitro modeling using this approach difficult and hampers its use in the evaluation of drug efficacy and toxicity.

**Conventional drug screening: a long, arduous road.** There has been a long list of high-profile drugs that have been withdrawn from the market because of their off-target and on-target toxicity to the heart, including the nonsteroidal anti-inflammatory drug rofecoxib (Vioxx, Merck & Co., Kenilworth, New Jersey), the gastrointestinal prokinetic drug cisapride (Propulsid; Johnson & Johnson, New Brunswick, New Jersey), and the broad-spectrum antibacterial drug grepafloxacin (Raxar, GlaxoSmithKline, Brentford, United Kingdom), all of which were withdrawn because of clinical cardiac or arrhythmogenic toxicities (161). With the pharmaceutical industry investing ~$2 billion per new drug over a period of 10 to 15 years (162), this situation creates a tremendous burden on the health-care system. The drug withdrawals are attributable in part to drug safety studies being evaluated in less than ideal nonhuman animal cells and models after years of laboratory and preclinical testing (Figure 2). The pharmaceutical industry typically conducts its cardiotoxicity studies of new drugs using in vitro cell lines, such as Chinese hamster ovary (CHO) cells and human embryonic kidney 293 (HEK293) cells overexpressing the $I_{Kr}$ protein hERG (human ether-a-go-go-related gene) (163,164). However, these in vitro cell lines do not replicate human CMs well, and the overexpression of a single cardiac ion channel in these cells does not recapitulate the complex channel biology in CMs. Because of these limitations, there is an inherent risk of cardiotoxic drugs slipping through initial screens only to be withdrawn from the market after patients have experienced harm and large investments have been incurred or wasted. Moreover, the converse might also be true. It is possible that pre-clinical testing of certain drugs using in vitro cell lines might show toxicity, only for the same drug to be later shown to be safe and efficacious for human usage (e.g., verapamil) (84,161). This could lead to valuable drugs not being marketed or developed at all because of inaccurate forecasting of their likely toxic effects by use of conventional approaches.

With all these known roadblocks in conventional drug screening, there is a compelling need to use functional human CMs for drug screening. PSC-CMs have now been comprehensively characterized as a good model for cardiotoxicity studies. Using a standard protocol, electrophysiological responses of PSC-CMs to a selection of known drugs that affect the hERG channel have shown comparable data to conventional hERG by use of in vitro assays (165,166). These studies indicate that PSC-CMs could be adopted as a standard model for cardiotoxicity studies. Importantly for pharmaceutical companies, this model could be a suitable addition to their conventional drug screening methods.

**iPSC-based drug screening: precise and personal.** Every patient has a unique genetic background and reacts differently to medications. Despite presenting with
similar symptoms, patients might have different underlying causes of the disease, but conventionally, they will still receive the same medication on the basis of their symptoms. iPSC-based drug screening technology might allow evaluation of a personalized therapy for every patient, an approach known as precision medicine.

With the advent of iPSCs and the improvements in differentiating iPSCs to functional CMs, it is now feasible to generate functional human CMs for drug screening, thereby avoiding the issues associated with earlier, less relevant screening systems and minimizing the time and cost for drug development. These ESC-CMs and iPSC-CMs have been thoroughly characterized to show similar electrophysiological, biochemical, and pharmacological functions that certify them as bona fide CMs.

In addition, PSC-CMs could be cultured in a dish for extended periods of time, and large-scale production of PSC-CMs is feasible from healthy control subjects or patients with various cardiac diseases (Table 1). This iPSC-based model could provide a valuable tool for the pre-clinical screening of candidates for whom a treatment would have therapeutic value and for screening candidates who might have off-target cardiac toxicity in any individual genotype, taking us one step closer to the goal of bringing precision medicine to the clinic (Figure 2).

Although PSC-CMs provide an excellent platform for drug screening and toxicology studies, they currently have several limitations. For example, PSC-CMs are generally immature and resemble fetal CMs both structurally and electrophysiologically. This cellular immaturity issue might seriously limit
drug development with PSCs. To overcome this limitation, several approaches have been developed to mature PSC-CMs, including differentiating them on 3-dimensional patches, which could enhance their contractile apparatus (120,168). Others have tried to achieve maturity by overexpressing microRNAs (miR-1/miR-499, Let7) to improve metabolic energetics (121,125) or by subjecting the cells to electrical and mechanical stimulation (169). In addition, uniaxial stretching of engineered heart muscle made from PSC-CMs could enhance their viability and maturation and has been used for drug screening (170). Despite their resemblance to fetal CMs and their labeling as “immature” CMs, these PSC-CMs have been used successfully to study drug efficacy and toxicity (84,85).

**IPSC CLINICAL TRIALS: TOWARD MACROMEDICINE.** As described previously, iPSC technology has revolutionized the concept of precision medicine. In the past, because of the lack of available human samples, drug toxicity screening was difficult and relied heavily on immortalized cell-based assay or in vivo animal models. Advances in iPSC technology could provide a steady supply of functional cells for pre-clinical screening of drugs. Moreover, iPSCs provide a unique platform that enables researchers to model diseases on a patient-by-patient basis. This micromedicine approach makes it possible to understand disease progression at an individual patient level and thus to screen for optimal pharmacological drugs individually. Beyond analyses performed for individual patients, high-throughput assays for drug toxicity that use human iPSC-CMs (171) can also be conducted, supporting the role that iPSC technology can play in macromedicine, under which iPSC-based medicine could be applied to cohorts of patients (172). In these clinical trials, iPSCs generated from patients could be differentiated into functional cells and then used to analyze the efficacy of drugs (Central Illustration). On this basis, patients could be classified as drug responders or nonresponders, and only those patients responding to the specific drugs would be moved to the next phase of the clinical trial. Thus, iPSCs could provide valuable patient stratification based on each patient’s responsiveness to the drug in clinical trials. Moreover, iPSCs could help us identify specific markers in the drug responders that correspond to important patient subpopulations, thereby boosting the success rate in clinical trials by pre-selecting patients who will benefit. Taken together, iPSC technology has the potential to significantly contribute to or even revolutionize macromedicine, for the first time allowing us to quickly and correctly identify a subset of patients with specific diseases who will respond to the drugs under study. Similarly, depending on the effectiveness of the drug in clinical trials, we might also gain the ability to diagnose sporadic diseases among a subset of patients. Given that iPSC-based clinical trials are in their infancy, much work is still needed to rapidly and economically develop personalized iPSCs.

**PRECISION MEDICINE: THE RIGHT DRUG FOR THE RIGHT PATIENT AT THE RIGHT DOSE.** The practice of medicine is undergoing a fundamental change. Rather than a generalized approach, the diagnostic and therapeutic strategies are now patient oriented, with a focus on precision medicine (30). This involves integrating the patient’s “omics” information (e.g., genomic, epigenomic, transcriptomic, and metabolomic) with advances in medical technology to tailor therapeutic options for each individual patient. With the advent of iPSC technology, this endeavor of precision medicine is now feasible.

There is an overwhelming consensus that cardiology will lead the development of precision medicine, after decades of research have revealed many of the molecular pathways that cause cardiomyopathies. Moreover, the use of iPSC-derived cardiovascular cells has now enabled researchers to understand the underlying mechanisms of many cardiac diseases that were difficult to model because of lack of patient samples. This new understanding has started to influence diagnostic and therapeutic strategies for cardiac diseases by the use of drugs that target specific molecular drivers. For example, the phenotype of iPSC-CMs derived from patients with long-QT syndrome was found to be affected by β-adrenergic receptor blockers (53). Similarly, a drug for malignant hyperthermia, dantrolene, was found to restore the abnormal Ca$^{2+}$ sparks and arrhythmogenic phenotype of iPSC-CMs derived from patients with catecholaminergic polymorphic ventricular tachycardia (81). Such genotype-guided therapy is precise and, if applied on a large scale, can transform patient care.

To achieve a deeper understanding of complex genetic cardiomyopathies, many more cardiac genomes need to be analyzed. This will require advances in medical technology that can help us better understand heart disease and that are capable of predicting an optimal treatment plan for each patient. With the availability of next-generation sequencing and bioinformatics, patient-specific iPSC-based pharmacogenomics can provide such valuable information. A patient’s iPSC-CMs could help identify the genetic basis of the disease, followed by disease modeling, finally leading to the identification of...
Induced pluripotent stem cell (iPSC) technology has contributed to both micromedicine and macromedicine. This includes everything from disease modeling and drug development based on cellular and molecular analyses of individual patients to iPSC clinical trials for stratification based on cellular and molecular analyses of a cohort of patients. Importantly, it could allow for the creation of a more precise clinical trial by identifying a subset of patients with a specific disease who optimally respond to the drugs under investigation, thereby boosting success rates by pre-selecting these drug responders. DNA = deoxyribonucleic acid; iPSC-CM = induced pluripotent stem cell-derived cardiomyocyte; RNA = ribonucleic acid.
pharmacogenetic biomarkers that can facilitate effective drug therapy. This information, once collected, can be matched with drug discovery screens to predict the most effective drug treatment (combination) for each person. In conclusion, patient-specific iPSC disease modeling and iPSC-based pharmacogenomics have the potential to identify patient-specific genetic loci that are responsible for disease and help develop the optimal individualized therapeutic strategy for each patient.

CONCLUSIONS AND FUTURE PERSPECTIVES

Since the initial basic discovery by Takahashi et al. (8,9) that a set of 4 transcription factors could reprogram mouse and human somatic cells to pluripotency, the field of iPSCs, free from the ethical issues associated with ESCs, has successfully expanded to different avenues of regenerative medicine. To date, iPSCs have 3 major applications, in disease modeling, drug discovery, and regenerative medicine (173). Human iPSC-CMs have been used extensively to characterize and model human heart diseases (174). There have been more than 700 publications in the past few years that have expanded their use to additional human diseases (both monogenic and polygenic). These advances in cardiac disease modeling have been made possible because of tissue-specific differentiation protocols that allowed researchers to derive healthy and disease-specific CMs (Table 1). In addition to disease modeling, several groups have tried to inject PSC-CMs into small and large animal models of myocardial infarction, with varying levels of success. Concerted efforts are now under way to further enhance the pre-clinical outcome of these PSC-CMs when transplanted into animal models of cardiac diseases. PSC-CMs also provide a unique platform to conduct drug-screening assays and to discover novel therapeutics. However, a major concern thus far is the immature status of the PSC-CMs. Various approaches, including cardiac tissue bioengineering, have been utilized to bring these PSC-CMs to maturity. Finally, the availability of genetically affected human tissues is very limited. Genome-edited PSC lines have now made it possible to recapitulate the human disease in a dish by use of various gene-editing tools, such as endonucleases (zinc finger nucleases or transcription activator-like effector nucleases) or palindromic repeats (clustered regularly-interspaced short palindromic repeat). Libraries of disease-specific cells can now be generated by creating allele-specific patient iPSC lines with which to conduct drug testing and toxicity studies. In summary, iPSC technology is not only revolutionizing science but will also fundamentally alter our healthcare system and the future of medicine by making it proactive, predictive, preventive, and personalized.

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