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Cardiomyocytes derived from human induced pluripotent stem cells as models for normal and diseased cardiac electrophysiology and contractility

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Abstract

Since the first description of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), these cells have garnered tremendous interest for their potential use in patient-specific analysis and therapy. Additionally, hiPSC-CMs can be derived from donor cells from patients with specific cardiac disorders, enabling *in vitro* human disease models for mechanistic study and therapeutic drug assessment. However, a full understanding of their electrophysiological and contractile function is necessary before this potential can be realized. Here, we review this emerging field from a functional perspective, with particular emphasis on beating rate, action potential, ionic currents, multicellular conduction, calcium handling and contraction. We further review extant hiPSC-CM disease models that recapitulate genetic myocardial disease.

Keywords

Human induced pluripotent stem cell; Cardiac cell; Embryoid body; Electrophysiology; Contraction; Optical mapping

1. Introduction

The successful derivation of human induced pluripotent stem cells (hiPSCs) in 2007 (Takahashi et al., 2007) and their differentiation into functional cardiomyocytes in 2009 (Zhang et al., 2009) has opened new horizons for the use of these cells as disease models and as platforms for drug discovery and toxicity screening [for reviews, see (Davis et al., 2011; Zeevi-Levin et al., 2012)]. Because cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) bear the genetic traits of the donors from whom they are obtained, they can serve as new experimental models to study the pathophysiological

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mechanisms of genetic diseases and to test personalized treatments (Dambrot et al., 2011; Josowitz et al., 2011). They are potentially also an unlimited source of cardiomyocytes for cardiac regeneration (Nsair and MacLellan, 2011), although the cost and time required to develop individualized therapy may be prohibitive factors (Freund and Mummery, 2009).

At present, hiPSCs and human embryonic stem cells (hESCs) are the two types of human pluripotent stem cells that can be differentiated in large numbers into working cardiomyocytes. hiPSC-CMs are particularly attractive for many investigators because they avoid most of the ethical concerns related with hESCs and can be derived in an autologous manner. In studies of cardiac genetic disease, they can potentially be used to supplant or replace animal models that only approximate human physiology or pathophysiology (Hamlin, 2007; McCauley and Wehrens, 2009; Milan and MacRae, 2005). These hiPSC-CMs can be subjected to detailed analysis of their molecular, pharmacological, electrophysiological and contractile properties [for reviews, see (Kong et al., 2010; Poon et al., 2011)], yielding valuable mechanistic insight.

However, if these cells are to reach their full potential, they must recapitulate the native physiological function of mature myocardial cells. For example, the utility of hiPSC-CMs from diseased patients lies in their *in vitro* expression of the functional hallmarks of the disease, so that the basic mechanisms of the disease or effects of disease-specific therapies can be studied. In this article, we review the current state of knowledge regarding the functional properties of hiPSC-CMs. While previous reviews have examined these characteristics largely from a genomic and differentiation perspective (Burridge et al., 2012; Josowitz et al., 2011), we focus here on fundamental contractile and electrophysiological properties. These are founded on sarcomere and myofilament organization, ion channel expression, calcium cycling proteins, and intercellular coupling. At a functional level, the properties include contractility, intracellular calcium release and uptake, action potentials, and drug responses, which together lead to physiological behavior that includes frequency-dependent changes in contraction and action potential duration, syncytial spread of electrical waves, and excitation-contraction coupling.

In the sections that follow, we summarize the known electrophysiological and contractile properties of hiPSC-CMs and, where applicable, compare them with cardiomyocytes derived from hESCs (hESC-CMs). We also review genetic cardiac diseases that have been modeled by hiPSC-CMs. Because the amount of information is currently rather limited, the extent to which the properties of hiPSC-CMs remain invariant, given the vast range of somatic cells from which hiPSCs can be reprogrammed, remains to be seen. The issue of a well-defined phenotype of the cells is an important consideration for the future usage of hiPSC-CMs and therefore, we address the challenges that exist in the development of hiPSC-CMs as models of physiological and pathophysiological function.

2. Beating rate

2.1. Hormonal responses

Beating rates in differentiated aggregates (termed embryoid bodies) derived from hiPSCs (hiPSC-EBs)[1], are often investigated as a first line of electrophysiological inquiry. In the adult heart, sinus rhythm is a process tightly regulated by the autonomic nervous system, and many studies have evaluated the response of hiPSC-CMs to adrenergic and cholinergic

[1]Note: Usage of the term embryoid body varies widely in the literature, ranging from natural aggregates of cells that co-assemble in hanging drops or other microenvironments, to dissected beating areas of the natural aggregates following plating on a dish. To avoid the clutter of detail, we have chosen to use the term loosely in this article to include both definitions, although in most cases it tends to be the latter. However, we will explicitly distinguish experiments on single hiPSC-CMs, hiPSC-CM monolayers and small cell aggregates of hiPSC-CMs.

stimulation. Isoproterenol, a β-adrenergic agonist known to increase sinus rate, increases the spontaneous beating rates in hiPSC-EBs (Fujiwara et al., 2011; Gupta et al., 2010; Mandel et al., 2012; Mehta et al., 2011), as well as monolayers of hiPSC-CMs (Guo et al., 2011a) in a dose-dependent manner, similar to findings in embryoid bodies from human embryonic stem cells (hESC-EBs) (Anderson et al., 2007; Gupta et al., 2010; Kapucu et al., 2008; Mandel et al., 2012; Pekkanen-Mattila et al., 2009; Reppel et al., 2004; Xu et al., 2006; Xu et al., 2002; Xu et al., 2009; Yokoo et al., 2009), dissociated hESC-CMs (Pekkanen-Mattila et al., 2009; Xu et al., 2006) and antibiotic selected hESC-CMs (Xu et al., 2009). Conversely, addition of the β-blocker propranolol prevented the positive chronotropic effect of isoproterenol (Mandel et al., 2012) and, when applied alone, decreased beating rate (Fujiwara et al., 2011). Carbachol, a cholinergic agonist, also decreased spontaneous beating rates in hiPSC-EBs (Gupta et al., 2010; Mandel et al., 2012).

2.2. Pharmacological responses

In addition to hormonal regulation, the role of specific ionic channels in regulating spontaneous beating rate has been investigated through the application of various pharmacological agents (see Section 3.2). The L-type calcium channel blocker verapamil was shown to decrease spontaneous beating rate of individual hiPSC-EBs in a dose-dependent manner and cease spontaneous beating at high concentrations (Mehta et al., 2011; Yokoo et al., 2009), similar to findings in hESC-EBs (Liang et al., 2010; Yokoo et al., 2009).

Conversely, the L-type calcium channel agonist Bay K 8644 has been shown to increase spontaneous beating rates in hiPSC-CMs (Mehta et al., 2011). Interestingly, the L-type calcium channel blocker nifedipine also increased beating rates in hiPSC-CMs (Guo et al., 2011a; Guo et al., 2011b). However, the nifedipine experiments were conducted using cryopreserved hiPSC-CMs, which may have different electrophysiological properties than those of fresh cells, given that cryopreservation has been shown to increase spontaneous beating rates in hESC-CMs (Kim et al., 2011b).

Blockade of the pacemaker current (I_f) with ZD 7288 (Guo et al., 2011a), potassium channels with amiodarone (Yokoo et al., 2009), or the rapid-delayed rectifier potassium current (I_{Kr}) with E-4031 (Guo et al., 2011a; Liang et al., 2010) all resulted in decreased beating rates in hiPSC-CMs. The responses to sodium channel (I_{Na}) blockers are more complex: TTX decreased spontaneous beating rate (Guo et al., 2011a) while procainamide, mexiletine and flecainide had no effect (Yokoo et al., 2009), perhaps due to differences in the specific mechanisms underlying the channel blockage or to multiple drug targets.

In one application of the cells, hiPSC-CMs were used for pharmacological screening and assessed for irregular spontaneous beating patterns reminiscent of arrhythmias (Guo et al., 2011a). Several cardioactive drugs known to block I_{Kr}, prolong the QT interval, or induce Torsades de Pointes (TdP, a life-threatening tachyarrhythmia characterized by an irregular rhythm) – including E-4031, RO5657, astemizole, cisapride, dofetilide, erythromycin, flecainide, quinidine, sotalol, terfenadine, and thioridazine – were applied to hiPSC-CMs and shown to induce dose- and time-dependent irregular spontaneous beating. In another study, hiPSC-CMs served as a model for studying cardiotoxicity and electrophysiological alteration caused by an oral drug, sunitinib (Cohen et al., 2011).

3. Electrophysiology

3.1. Action Potentials

3.1.1. Microelectrode and patch clamp recordings—The properties of the cardiac action potential (AP) enable proper cardiac electrical and contractile function. Slow, regular

depolarization from rest is necessary in the nodal regions of the heart as the basis for automaticity, while fast upstroke velocities and long action potential durations (APDs) allow fast propagation and calcium influx for contraction in the ventricles. An understanding of hiPSC-CM AP properties is, therefore, critical for an accurate assessment of the functional maturity of the cells. APs have been recorded from single hiPSC-CMs (Burridge et al., 2011; Fujiwara et al., 2011; Haase et al., 2009; Lee et al., 2011; Ma et al., 2011; Novak et al., 2010; Ren et al., 2011; Zhang et al., 2009) and in clusters of hiPSC-CMs (Burridge et al., 2011; Fujiwara et al., 2011; Haase et al., 2009; Lee et al., 2011; Ma et al., 2011; Novak et al., 2010; Ren et al., 2011; Zhang et al., 2009). In agreement with the hESC-CM literature, three AP phenotypes (nodal-, atrial-, or ventricular-like)(Fujiwara et al., 2011; Haase et al., 2009; Lee et al., 2011; Ma et al., 2011; Zhang et al., 2009) have generally been identified (Table 1). The percentages of each phenotype clearly depend on the selection criteria, which are not always well-defined, although in some cases quantitative measures have been used, such as the ratio of APD at different percentages of repolarization (Ma et al., 2011).

A general finding is that a single AP phenotype predominates over others within a single EB (Zhang et al., 2009). This is in agreement with similar studies in hESC-CMs (He et al., 2003; Magyar et al., 2000). Across different cell lines, the percentages of electrophysiological phenotypes vary, with ventricular-like cells ranging from 38%–54% when measured in isolated cells (Lee et al., 2011; Ma et al., 2011), to 70–74% in EBs (Zhang et al., 2009). These percentages are similar to those in hESC-CMs, and to optical mapping measurements from our labs that found predominantly ventricular-like potentials for both hESC-CMs and hiPSC-CMs (Burridge et al., 2011).

Studies in both hiPSC-CMs (Fujiwara et al., 2011; Haase et al., 2009) and hiPSC-EBs (Zhang et al., 2009) reported ventricular-like action potentials with maximum diastolic potential (MDP) values near −60 mV. However, a more recent report in a blasticidin-enriched population of single cells reported a value of −75 mV (Ma et al., 2011), closer to the −87 mV resting potential of adult ventricular cells (Drouin et al., 1995). hiPSC-CM ventricular action potential amplitude (APA) in these studies ranged from 85–105 mV. Maximum upstroke velocity (dV/dt_{max}) and APD at 90% repolarization have ranges of 10–40 V/s and 300–400 ms respectively (Haase et al., 2009; Ma et al., 2011; Zhang et al., 2009). Interestingly, isolated cells with very negative MDP values have been reported to have low dV/dt_{max}(27.8±4.8 V/s) (Ma et al., 2011). There was no significant difference in differentiation time between these studies to account for the wide range of values, but each used a different cell source (fetal or newborn fibroblasts and cord blood endothelial cells) and, sometimes, a cardiomyocyte enrichment process. As a further illustration of hiPSC-CM functionality, APD decreases with increasing stimulation rates (1–3 Hz) (Zhang et al., 2009). In general the AP properties of hiPSC-CMs are similar to those of the more extensively studied hESC-CMs and are those of immature cardiac cells (low resting potential, slow upstroke velocity, and diastolic depolarization).

Pharmacologically, application of TTX to isolated cells delayed AP upstroke and decreased dV/dt_{max}(Ma et al., 2011). Nifedipine decreased isolated cell APDs at 10, 50, and 90% repolarization (Ma et al., 2011), while E-4031 increased APD in a dose-dependent manner (Fujiwara et al., 2011; Ma et al., 2011; Moretti et al., 2010). Chromanol 293B, a blocker of slow delayed rectifier current I_{Ks}, did not cause significant APD changes (Ma et al., 2011).

3.1.2. Field potentials—Extracellular field potentials measured by microelectrode arrays (MEAs) can serve as a surrogate for AP measurements in multicellular preparations (Halbach et al., 2003). The amplitude of the first negative peak of the field potential has been used as a measure of dV/dt_{max}, and was found to decrease with TTX (Mehta et al., 2011). Field potential duration (FPD) directly correlates with APD in cardiomyocytes and

the QT interval of an electrocardiogram, and its response to various ion channel blockers has been measured in hiPSC-EBs or monolayers of hiPSC-CMs. Consistently, FPD is prolonged by E-4031 (Braam et al., 2010; Caspi et al., 2009; Fujiwara et al., 2011; Mehta et al., 2011) and shortened by verapamil (Braam et al., 2010; Liang et al., 2010; Mehta et al., 2011) and nifedipine (Braam et al., 2010; Guo et al., 2011b).

3.1.3. Optical mapping—Optical mapping is a technique in which stem cell-derived cardiomyocytes can be stained with a fluorescent dye and, upon illumination, emit a fluorescent signal related to the activity of the cell (Weinberg et al., 2010). We recently used optical mapping to measure the transmembrane voltage and intracellular calcium levels of cell aggregates mechanically dissected from hiPSC-EBs derived from cord blood cells (Burridge et al., 2011). Optical recordings of transmembrane potential (V_m) or intracellular calcium (Ca_i) were recorded using an electron multiplying charged coupled device (EMCCD) camera at 490 frames per second. In V_m recordings, we found that electrical waves propagated across the hiPSC-EB, demonstrating a functional syncytium (Figure 1 A a–c). Additionally, in Ca_i recordings, intracellular calcium transients were present in the hiPSC-EB, which also propagated across the EB as calcium waves (Figure 1 B). In a study from another group, APDs of hiPSC-EBs were found to be predominantly ventricular-like, prolonged by procainamide (sodium and potassium channel blocker) and shortened by verapamil (Ren et al., 2011).

3.2. Ionic currents

Recent studies have characterized the biophysical properties of the ionic currents I_{Na}, I_{CaL}, I_{K1}, I_{to}, I_{Kr}, I_{Ks}, *and* I_f in hiPSC-CMs. Gene expression data of the corresponding ion channels are summarized in Table 2, although in no case were data in this table evaluated for a specific electrophysiological phenotype. These data demonstrate that the major currents responsible for the AP in native cardiomyocytes are genetically expressed and that their gene expression increases with differentiation time.

The presence and upregulation of these cardiac ion channel genes has led to investigations of the functional presence of their associated currents. Each of the currents in Table 2 has been identified functionally in hiPSC-CMs by voltage- and current-clamp experiments that are summarized below. However, the currents have not yet been studied for specific AP phenotypes. In many cases direct comparison of currents is confounded by different voltage protocols or other experimental conditions between studies.

Voltage clamp separation of the fast sodium current demonstrated that I_{Na} has a maximal value of −216 pA/pF at −20 mV (Ma et al., 2011), in close agreement with previous hESC-CM measurements (Satin et al., 2004). For comparison, I_{Na} of adult human ventricular cardiomyocytes can be estimated from the O'Hara computational model (O'Hara et al., 2011) to have a peak amplitude of −196 pA/pF at −17 mV under physiological conditions.

Peak L-type calcium current, I_{CaL}, of −17 pA/pF at 0 mV (with 5 mM extracellular Ca^{2+}) has been reported via voltage clamp separation (Ma et al., 2011) and is about four times larger than that reported in hESC-CMs (with 1.8 mM extracellular Ca^{2+}) (Sartiani et al., 2007). The same hiPSC-CM study also noted an absence of T-type calcium currents. In human ventricular cells from children peak I_{CaL} is approximately −5.8 pA/pF at +10 mV (Pelzmann et al., 1998). A recent study showed that, in comparison to native guinea pig ventricular myocytes, hiPSC-CMs maintained similar kinetic responses to antagonists, but different responses to activators (Kang et al., 2012).

The inward rectifier potassium current, I_{K1}, has not generally been found in hiPSC-CMs, similar to studies in hESC-CMs (Fu et al., 2010; Fu et al., 2011; He et al., 2003; Satin et al.,

2004). However, one study reported I_{K1} as a Ba^{2+}-sensitive current with an amplitude of −2.3 pA/pF at −123 mV (Ma et al., 2011), less than that in adult ventricular cells (−10 pA/pF at −90 mV) (Wang et al., 1998). This is near the value reported in hESC-CMs that were differentiated for 50–110 days (Sartiani et al., 2007). The same study also reported an MDP value of −75 mV, the most negative to date in hiPSC-CMs, suggesting that I_{K1} is functionally active. The hiPSC-CMs used in that study were highly purified by exploiting blasticidin resistance under the control of the α-myosin heavy chain promoter, which may contribute to a more mature phenotype.

The transient outward potassium current, I_{to}, was measured by voltage clamp separation and has a reported density of 2.5 pA/pF at 60 mV (Ma et al., 2011). This value is lower than that reported in hESC-CMs (6 pA/pF at 50 mV) (Fu et al., 2011), but is within the range of values reported in adult ventricular cells (2.3±0.3 to 7.9±0.7 pA/pF at 60 mV for subepicardial and subendocardial cells, respectively) (Wettwer et al., 1994).

The rapid delayed rectifier current, I_{Kr}, has been measured as an E-4031-sensitive current with a maximal current of 0.4 pA/pF at approximately −10 mV (Ma et al., 2011). This magnitude agrees closely with that in hESC-CMs, but it occurs at a more negative voltage (Fu et al., 2011; Li et al., 1996; Wang et al., 2011). In adult ventricular cardiomyocytes, I_{Kr} can be determined from the O'Hara model to have a magnitude of 0.82 pA/pF at +7 mV under physiological conditions.

The slow delayed rectifier current, I_{Ks}, is functionally expressed in single hiPSC-CMs (Ma et al., 2011; Moretti et al., 2010; Wang et al., 2011), as it is in hESC-CMs (Wang et al., 2011). It was measured as a chromanol 293B-sensitive current and, when present, had a relatively small magnitude (0.31 pA/pF at 40 mV in 5 of 16 cardiomyocytes studied) (Ma et al., 2011). In adult ventricular cardiomyocytes, I_{Ks} can be determined from the O'Hara model to have a magnitude of 0.78 pA/pF at +40 mV under physiological conditions.

The pacemaker current, I_f, has been measured in single cells via voltage clamp separation and has a control value of −4.1+/−0.3 pA/pF at −120 mV (Ma et al., 2011). This is smaller in magnitude than the value reported in adult sinoatrial node cells (−7 pA/pF at −120 mV) (Verkerk et al., 2007). The I_f blockers ZD7288 and CsCl each significantly decreased I_f current density in hiPSC-CMs.

3.3. Cell-cell coupling

Cell-cell coupling provides a mechanism for direct electrical connections between neighboring cardiomyocytes within an hiPSC-EB. Synchronous beating across the hiPSC-EB provides visual evidence of cell-cell coupling, although it may be difficult to distinguish whether it is the result of electrical synchrony of activation or the mechanical pulling of one cell on adjacent cells. The following studies have provided evidence for direct electrical cell-cell coupling of cardiomyocytes within hiPSC-EBs.

3.3.1. Gap junctions—The presence of gap junctions among hiPSC-CMs has been demonstrated in several studies by immunofluorescence imaging (Haase et al., 2009; Martinez-Fernandez et al., 2009; Zwi et al., 2009). However, one study using electron microscopy was unable to find gap junctions in hiPSC-EBs after 38 days of differentiation (Gherghiceanu et al., 2011).

3.3.2. Conduction—Apart from the presence of junctional coupling proteins, a functional syncytium is ultimately demonstrated by the occurrence of electrical conduction. One study of spontaneously beating hiPSC-EBs reported conduction velocity measurements of 1–2 cm/s at physiological temperatures (Mehta et al., 2011). hiPSC-EBs respond to a range of

cardioactive compounds. For example, TTX slows conduction in a dose-dependent manner (Mehta et al., 2011), suggesting a Na^+ current-driven conduction similar to that in adult myocardium. In optical mapping experiments from our labs (Burridge et al., 2011), conduction velocities in EBs were on average ~ 1 cm/s and increased with the addition of isoproterenol (Figure 1 A d).

4. Contractility

4.1. Contraction and force generation

Immunofluorescence studies have demonstrated the presence of organized structural and contractile proteins forming sarcomere patterns in hiPSC-CMs (Figure 2) (Burridge et al., 2011; Fujiwara et al., 2011; Gai et al., 2009; Germanguz et al., 2011; Gupta et al., 2010; Haase et al., 2009; Itzhaki et al., 2011b; Lee et al., 2011; Novak et al., 2010; Ren et al., 2011; Tulloch et al., 2011; Yokoo et al., 2009; Zhang et al., 2009; Zwi et al., 2009), similar to previous findings in hESC-CMs. Studies have also shown that hiPSC-CMs contract in response to electrical stimulation (Germanguz et al., 2011; Novak et al., 2010). One study showed that block of L-type calcium channels decreased the amplitude of contraction in a dose-dependent manner, like that seen in hESC-CMs (Yokoo et al., 2009). Similar to observations in hESC-CMs, the addition of isoproterenol increased the amplitude and rate of contraction in hiPSC-CMs (Germanguz et al., 2011; Novak et al., 2010; Yokoo et al., 2009), with a greater increase in contraction from isoproterenol observed in later stage (18–70 day) cells compared with earlier stage (10–15 day) cells (Germanguz et al., 2011).

A negative force-frequency relation (rate-staircase) of contraction was measured in small aggregates of hiPSC-CMs (Germanguz et al., 2011), similar to that observed for hESC-CMs in the same study but unlike the positive force-frequency relation characteristic of native myocardium (Endoh, 2004). However, the same study also showed weak post-rest potentiation of contraction, suggesting some accumulation of Ca^{2+} stores. Furthermore, ryanodine decreased peak contraction amplitude and maximum rates of contraction and relaxation of hiPSC-EBs, but had no effect on hESC-EBs. These findings suggest functional but immature calcium cycling in hiPSC-CMs.

4.2. Calcium cycling

Recently, much effort has gone into the characterization of calcium cycling in hiPSC-CMs and, in some studies, comparisons with hESC-CMs. Expression of key calcium cycling proteins or their gene products has been demonstrated (Table 3): $Ca_v1.2$, RyR2, SERCA2a, and IP3R2, as well as of the regulatory proteins calsequestrin (CSQ) and phospholamban (PLN) (Itzhaki et al., 2011b). However, in no case were these expression data evaluated for a specific cell pheonotype. One study compared cardiomyocytes derived from two hiPSC cell lines with those from two hESC cell lines, and expression of RyR2 and SERCA2a was significantly lower in the hiPSC-CMs (Lee et al., 2011). In another study, protein expression of RyR and CSQ was obtained in hiPSC-CMs (Germanguz et al., 2011).

Functional calcium transients are present in hiPSC-CMs and can be used to assess calcium release and uptake from the sarcoplasmic reticulum (SR), although in no case were these SR properties evaluated for a specific electrophysiological phenotype (Table 4). One study found that calcium transients could be abolished by blocking calcium influx through the L-type calcium channel, calcium release via the RyR release channel, or calcium uptake via the SERCA pump (Itzhaki et al., 2011b). Comparative studies of hiPSC-CMs with hESC-CMs revealed smaller calcium transient amplitudes and slower upstroke and decay rates in hiPSC-CMs, suggesting less mature calcium cycling in hiPSC-CMs (Lee et al., 2011). Partial block of RyR release reduced the calcium transient amplitude and slowed the upstroke rate in hiPSC-CMs, but to a lesser extent than in hESC-CMs. This indicates that

calcium cycling might not be operational in hiPSC-CMs, contrary to observations in hESC-CMs (Lee et al., 2011) and contradicting the contraction experiments reported in Section 4.1.

Addition of the β-adrenergic agonist isoproterenol, known to increase L-type calcium channel current and SR calcium release (Eisner et al., 2009), increased the amplitude of calcium transients (Novak et al., 2010; Ren et al., 2011).

As with hESC-CMs, hiPSC-CMs exhibit SR calcium release sensitivity to large doses of caffeine, revealing the presence of functional SR calcium stores (Germanguz et al., 2011; Gupta et al., 2010; Itzhaki et al., 2011b; Lee et al., 2011). One study showed reduced calcium release and slower release rate following caffeine in hiPSC-CMs compared with hESC-CMs, along with U-shaped calcium waves attributable to the absence of T-tubules in both hESC-CMs and hiPSC-CMs (Lee et al., 2011). The delay was greater for hiPSC-CMs, consistent with reduced expression of triadin (Lee et al., 2011). In line with the latter findings, a study using electron microscopy failed to find T-tubules in hIPSC-CMs (Gherghiceanu et al., 2011), although that study also found poorly developed SR in contrast with the prior mentioned studies.

Additionally, SR calcium stores were recently shown to regulate the spontaneous beating of hiPSC-CMs. Blocking either RyR or IP3R calcium release decreased the spontaneous beating rate (Itzhaki et al., 2011b), similar to results in hESC-CMs (Kim et al., 2010a) and consistent with the proposed “calcium-clock” mechanism for spontaneous sinus rhythm (Maltsev and Lakatta, 2007).

5. Disease models

In order to provide mechanistic insights into cardiac genetic diseases or serve as patient-specific cardiac drug testing platforms, hiPSC-CMs models must faithfully recapitulate the disease phenotype and pathoelectrophysiology. Unlike hESC-CM-based disease models which can only be created for known mutations that are introduced into the cells (Maury et al., 2012), hiPSC-CMs can be produced from patient-derived somatic cells and will retain the genetic abnormalities associated with the patient’s condition. Several cardiac disease models have been established with hiPSC-CMs and demonstrate the ability to recapitulate the cellular pathogenic hallmarks of the diseases.

5.1. LEOPARD Syndrome

LEOPARD syndrome is an autosomal-dominant developmental disorder that involves abnormal RAS-mitogen-activated protein kinase (RAS-MAPK) signaling (Gorlin et al., 1971; Sarkozy et al., 2008). In 90% of cases, it is caused by a missense mutation in the *PTPN11* gene encoding the protein tyrosine phosphatase SHP2, which has the capacity to enhance activation of RAS-MAPK signaling (Digilio et al., 2002; Edouard et al., 2007). Left ventricular hypertrophy is the most common cardiac anomaly (Limongelli et al., 2007).

Dermal fibroblasts were reprogrammed from LEOPARD syndrome patients having heterozygous T468M substitution mutations in *PTPN11* (Carvajal-Vergara et al., 2010) and then differentiated into cardiomyocytes. The hiPSC-CMs that were generated exhibited increased cell size and sarcomeric organization, suggestive of the cardiac hypertrophic response, as well as aberrant RAS-MAPK signaling. One limitation of the model is that cardiac differentiation from hiPSCs was performed by standard EB culture, resulting in a heterogeneous population of cells. As noted by the study investigators, in the absence of a purified cardiomyocyte population, protein synthesis rates and fetal gene program reactivation could not be used to assess cardiomyocyte hypertrophy.

5.2. Long QT Syndrome

Long QT syndrome (LQTS) is a congenital cardiovascular disorder characterized by prolongation of the QT interval and syncopal episodes due to polymorphic ventricular tachycardia that may lead to cardiac arrest and sudden cardiac death (Crotti et al., 2008). The syndrome can be caused by a variety of mutations in genes encoding cardiac ion channels, with the most common types, LQT1 and LQT2, occurring from mutations in *KCNQ1* and *KCNH2*. These genes encode the alpha subunits of potassium channels that conduct I_{Ks} and I_{Kr}, respectively (Josowitz et al., 2011; Marban, 2002). Studying LQTS is of interest because of its prevalence [estimated at 1 in 2,500 live births (Crotti et al., 2008)] and because measures such as AP prolongation can be used to screen potential therapeutics.

iPSCs were generated by reprogramming dermal fibroblasts from patients with a heterozygous R190Q missense mutation in the *KCNQ1* gene (Moretti et al., 2010). These LQT1 hiPSC-CMs could be classified as ventricular-, atrial-, or nodal-like based on AP phenotype (see Section 3.1.1) and specific myocyte-lineage markers that distinguish between these phenotypes. Ventricular- and atrial-like hiPSC-CMs exhibited longer APs, slower repolarization, and decreased APD shortening in response to higher pacing frequencies (decreased APD restitution slope) compared with ventricular- and atrial-like hiPSC-CMs from healthy control subjects. Immunocytochemical studies of *KCNQ1* suggested a trafficking defect in these cells, while whole-cell patch clamping indicated a 75% decrease in I_{Ks} conductance along with altered I_{Ks} activation and inactivation. In spontaneously beating LQT1 hiPSC-CMs, adrenergic stimulation induced early afterdepolarizations (EADs), affecting approximately 9% of beats. Pretreatment with a β-blocker decreased the effect of adrenergic stimulation, resulting in EADs that affected about 2% of beats.

hiPSC-CMs have been derived from LQT2 patients with a A614V (Itzhaki et al., 2011a), G1681A (Matsa et al., 2011), or R176W (Lahti et al., 2011) mutation in the *KCNH2* gene. In all three models, atrial- and ventricular-like LQT2 hiPSC-CMs had prolonged APDs compared with control hiPSC-CMs derived from healthy human (Itzhaki et al., 2011a; Lahti et al., 2011) or from hESC-derived fibroblasts (Matsa et al., 2011). However, in the case of the R176W mutant cells, this prolongation was only statistically significant for ventricular-like cells, and nodal-like APDs were not observed (Lahti et al., 2011). With the A614V and G1681A mutants, APDs in nodal-like hiPSC-CMs were not significantly prolonged in the LQT2 model compared with controls (Itzhaki et al., 2011a; Matsa et al., 2011). Multicellular clusters of LQT2 hiPSC-CMs, in the form of hEBs (Itzhaki et al., 2011a; Matsa et al., 2011) or spontaneously beating cardiomyocyte aggregates excised from END-2 co-culture (Lahti et al., 2011), exhibited prolonged FPD compared with healthy hiPSC-CM controls (Itzhaki et al., 2011a; Lahti et al., 2011; Matsa et al., 2011). The relative ventricular:atrial:nodal-like composition of these clusters was not specified. In the A614V model, hiPSC-CMs in hEBs developed both single and sustained EADs, as well as ectopic activity (Itzhaki et al., 2011a). EADs were also elicited in the G1681A model with the application of adrenergic stimulation, along with a greater reduction in single-cell APD relative to controls (Matsa et al., 2011). Sotalol elicited EADs in the R176W modeI at the highest concentrations tested (Lahti et al., 2011). In all of the models, blocking I_{Kr} through the addition of E4031 caused APD prolongation and evoked new or increased the number of existing EADs. I_{Kr} was found to be reduced in the A614V and R176W models but was not measured in the G1681A model (Itzhaki et al., 2011a; Lahti et al., 2011). Potassium channel openers (Itzhaki et al., 2011a; Matsa et al., 2011) or an L-type Ca channel blocker (Itzhaki et al., 2011a) decreased APD in the A614V and G1681A models. In G1681A hiPSC-CMs, treatment with potassium channel activators in combination with adrenergic stimulation evoked EADs (Matsa et al., 2011).

5.3. Timothy Syndrome

Timothy syndrome is a multisystem disorder that results in syncope and severe cardiac arrhythmias that can lead to sudden cardiac death (Splawski et al., 2005). It is caused by a mutation of the *CACNA1C* gene encoding $Ca_V1.2$, the alpha 1C subunit of the L-type Ca channel. This results in maintained inward Ca^{2+} currents due to loss of voltage-dependent Ca^{2+} channel inactivation (Splawski et al., 2004). HiPSC-CMs have been derived from fibroblasts of Timothy syndrome patients with a G406R mutation in exon 8a of the *CACNA1C* gene.(Yazawa et al., 2011). Action potentials of ventricular Timothy syndrome hiPSC-CMs were three times longer than those of control hiPSC-CMs from healthy individuals, and voltage-dependent inactivation of I_{CaL} was reduced compared with controls. These cells also exhibited a large number of delayed afterdepolarization-like events. Additionally, the intracellular Ca^{2+} transients were irregular in timing and amplitude, exhibiting larger and more prolonged elevations compared with controls, consistent with delayed $Ca_V1.2$ inactivation. Application of the cyclin-dependent kinase inhibitor roscovitine reduced irregularities in the timing and amplitude of Ca^{2+} transients, reduced APD, and decreased the frequency of abnormal depolarizing events.

5.4. Catecholaminergic Polymorphic Ventricular Tachycardia

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmogenic disorder that can result in sudden cardiac death and is caused by mutations in the calsequestrin (*CASQ2*) gene or in the *RyR2* gene encoding the cardiac isoform of the ryanodine receptor (di Barletta et al., 2006). hiPSC-CMs have been derived from dermal fibroblasts of CPVT patients with a D307H mutation of the *CASQ2* gene (Novak et al., 2011) and from a patient with a S4061L mutation in the *RyR2* gene (Jung et al., 2012). Treatment of the mutant *CASQ2* hiPSC-CMs with isoproterenol induced after-contractions and triggered activity concurrent with a rise in resting tension level, as well as delayed afterdepolarizations and late diastolic oscillations (Novak et al., 2011). Unlike control hiPSC-CMs, contraction amplitude, maximal contraction rate, and relaxation rate were all unaffected by isoproterenol in CPVT hiPSC-CMs. APD at 50% repolarization was nearly twice as long in CPVT cells, but other parameters (APA, dV/dt_{max}) were similar. Myofibrillar structure was less organized, and fewer caveolae were found in CPVT versus control hiPSC-CMs (Novak et al., 2011).

In the mutant *RyR2* hiPSC-CMs, delayed afterdepolarizations and triggered activity were also observed in a majority of ventricular- and atrial-like hiPSC-CMs during pacing (Jung et al., 2012). Furthermore, the incidence of spontaneous APs after pacing termination was higher for the mutant hiPSC-CMs when compared with controls. In mutant *RyR2* hiPSC-CMs, abnormal calcium handling (indicated by Ca^{2+} alternans), absence of triggered Ca^{2+} transients on successive beats, and irregular Ca^{2+} oscillations were observed in a higher number of cells compared with controls during increasing stimulation frequency. Application of isoproterenol resulted in elevated diastolic Ca^{2+} and failed to increase SR Ca^{2+} load in CPVT cells compared with controls, suggesting diastolic Ca^{2+} leak from the SR. Ca^{2+} sparks measured in CPVT had a greater amplitude, full width at 50% peak amplitude, and decay than healthy control cells. Furthermore, application of isoproterenol caused an increase in Ca^{2+} spark frequency, decay time constant, and duration of abnormal sparks compared with controls. The observed Ca^{2+} spark properties were present in both ventricular and non-ventricular CPVT hiPSC-CMs. The disease phenotype, including abnormal Ca^{2+} properties, Ca^{2+} handling during catecholaminergic challenge, delayed afterdepolarizations, and triggered activity, was rescued by the application of the drug dantrolene.

6. Future Challenges

6.1. Deriving large-scale amounts of clinically useful cardiomyocytes for regenerative medicine

Although many published studies have demonstrated the ability of pluripotent stem cells to differentiate into cardiomyocytes (Burridge et al., 2007; Gai et al., 2009; Habib et al., 2008; Laflamme et al., 2007; Mummery et al., 2003; Zhang et al., 2009; Zwi et al., 2009), the current inefficiency of these systems makes the massive scale of cardiomyocytes needed to replace the cells from a single patient's myocardial infarction (MI) impractical. For example, it has been estimated that one billion cardiomyocytes are lost in a typical MI, and over one hundred 10 cm dishes of cells would be required to replace these infarcted cells. This task requires a substantial investment in increasing the efficiency and scalability of obtaining hiPSC-derived cardiomyocytes, which is significantly lower than those of hESCs for which current hESC differentiation protocols typically produce only rare amounts of beating embryoid body cells. The second major limitation involves the technical caveats (and safety risks) of deriving hiPSC with viral vectors. Despite overall silencing of integrated retroviral and lentivector promoters during iPSC generation, low levels of viral transgenes or reactivated vector promoters result in incompletely reprogrammed states that promote insertional mutagenesis, or even malignant transformation (Miura et al., 2009; Nakagawa et al., 2008). Alternative approaches for generating iPSC are needed that reduce these risks by avoiding either the use of stably integrating sequences or potentially mutagenic chromatin-modifying molecules to enhance reprogramming (Burridge et al., 2011; Jia et al., 2010; Okita et al., 2008; Stadtfeld et al., 2008; Warren et al., 2010; Yu et al., 2009; Zhou et al., 2009). Much investment in this high priority area of research is still needed.

6.2. Developing faithful hiPSC models of cardiac disease

The use of iPSCs for applications in disease modeling is now advancing markedly. However, despite the rapid pace of improvement in cardiac differentiation methods, and development of new hiPSC lines harboring cardiac disease phenotypes, significant challenges still remain. For example, there are several important aspects relating to the generation of hiPSCs that impact the faithful recapitulation of the electrophysiological cardiac disease phenotype. These include the developmental maturity, and the retention of somatic epigenetic memory of hiPSCs. Thus far, the cardiomyocytes that have been generated from hiPSCs display an immature electrophysiological and contractile phenotype (see Sections 3.1.1, 4.1 and 4.2). These cardiomyocytes also displayed spontaneous contractions, embryonic cardiac gene expression profiles, and fetal type ion channels. It is unclear at this time if these developmentally immature phenotypes will pose a limitation for downstream drug testing and disease modeling applications. Furthermore, the generation of immature cardiomyocytes with automaticity will likely pose a challenge for ultimate application in regenerative medicine. It is not clear if these cardiomyocytes will engraft robustly in damaged adult myocardium environment with a vastly incongruent physiological status. On the other hand, there is also the possibility that developmentally immature cardiomyocytes may alternatively display an *augmented* long term survival in an adult environment, since rat fetal cardiomyocytes engraft more potently in rat adult myocardium (Reinecke et al., 1999). Approaches will be needed to mature fetal-like hiPSC-CMs further, much like those that are being developed for hESC-CMs (Magyar et al., 2000), and the detailed electrophysiologic characterization using the methods we have reviewed herein will be vital in solving this critical caveat.

An important caveat, that our group has specifically invested effort into solving, is the obstacle of interline variability in cardiac differentiation that exists in hiPSC and hESC lines

(Burridge et al., 2011). Although various hESC lines have been documented to have a greater propensity for cardiac differentiation (Burridge et al., 2007; Osafune et al., 2008; Pal et al., 2009; Pekkanen-Mattila et al., 2009), this variability appears to be even more pronounced for hiPSC (Narsinh et al., 2011). The various factors that likely contribute to this efficiency in cardiac differentiation include the protocol used for cardiac induction, the somatic source used for reprogramming (e.g. fibroblasts vs blood cells) (Kim et al., 2010b; Kim et al., 2012; Polo et al., 2010), the expression of endogenous mesoderm-inducing factors in various pluripotent lines, and the retention of somatic memory of the reprogrammed donor cell, which may inherently prevent differentiation across lineages (Kim et al., 2010b; Kim et al., 2012) (for example from fibroblast to neural or cardiac lineages). Retention of somatic epigenetic memory was initially thought to be restricted to incompletely reprogrammed, low passage hiPSCs. However, further work has revealed that even after prolonged passages, there are quantitative losses in differentiation potential of hiPSCs in comparison to hESCs (Kim et al., 2011a; Polo et al., 2010). More refined cardiac differentiation protocols (Burridge et al., 2011), as well as improved methods of reprogramming that produce hiPSC with minimal epigenetic memory may overcome these obstacles in the near future.

6.3. Developing hiPSC models of drug discovery

The efficient *in vitro* production of human cardiomyocytes from induced pluripotent stem cells also opens unique opportunities for deriving genetically-defined cells for drug discovery applications (Yokoo et al., 2009); for reviews, see (Davis et al., 2011; Zeevi-Levin et al., 2012). However, discovery, development, and safety testing of novel drugs is a long, arduous and expensive process that is complicated by expensive and unreliable methods for accurately modeling human cardiac electrophysiology. The pharmaceutical industry currently invests ~$1.5 billion to take a candidate drug from primary screen to market, and many drugs are withdrawn due to side effects associated with electrophysiological alterations. For example, the anti-inflammatory drug Vioxx and the gastrointestinal prokinetic drug Cisapride were both withdrawn following unanticipated arrhythmogenic toxicities (Fermini and Fossa, 2003). Off-target cardiac toxicity is also the most common cause of regulatory delays in approvals or market withdrawals of new pharmaceuticals. Current barriers that impede drug discovery and development include: 1) the use of non-translatable nonhuman animal models for assessment of off-target toxicity; 2) the performance of early compound safety screening studies with quantities too small for scale-up to animal models; 3) the small scale of human clinical trials (usually 20–50 patients) that do not include critically relevant genetic backgrounds.

The use of hiPSC-derived cardiomyocytes provides the potential to reduce the burden of each of these factors, and therefore reduce the time and cost of bringing new drugs to market. As outlined above, cardiomyocytes display comparably normal cardiac molecular, structural and functional characteristics, survive under cell culture conditions for extended periods, and can be grown in controllable environmental conditions. Thus, large-scale generation of cardiomyocytes from normal or heart disease-affected hiPSCs, holds the potential to serve as a human-based model for both drug development and cardiotoxicity screening and would provide the pharmaceutical industry with an invaluable tool for pre-clinical screening of candidate anti-arrhythmic, anti-heart failure, and other medicines for secondary cardiac toxicities (*e.g.* chemotherapeutics; (Farokhpour et al., 2009)).

The effects of drugs on pluripotent stem cell-derived cardiomyocytes could potentially be assayed using simple ‘number of beating hEB’ assays (Burridge et al., 2011; Farokhpour et al., 2009) or more detailed studies using current clamp (Chaudhary et al., 2006), patch-clamp, laser capture microscopy (Chaudhary et al., 2006), or MEAs (Tanaka et al., 2009).

Both automated robotic high-throughput patch-clamp systems and MEA systems enabling high throughput screening of 96 or even 384 compounds simultaneously are already commercially available. These systems enable the measurement of the surface electrogenic activities of cell clusters and allow stable, long-duration recordings that are necessary to evaluate the relationships between dose-dependency and the inductive side-effects of new drugs. Since the washing out of drugs can lead to the recovery of the field potential waveform to the baseline observed before the drug application, such systems can test the reproducibility of pharmacological effects of multiple drugs on the same sample. Ultimately, the use of hiPSC-CMs will allow the possibility of drug testing on a panel of cell lines that might more closely reflect the genetic diversity of a population, such as is currently performed using murine ESCs (McNeish, 2004). Genetic conditions that affect the heart such as familial cardiomyopathy, familial lethal arrythmias and congenital heart diseases, as reviewed above, can further be studied with appropriate disease-harboring hiPSCs.

7. Conclusion

Despite their relatively recent discovery, the electrophysiological and contractile function of hiPSC-CMs has been the subject of significant research investigation. To date, their beating rates, action potentials, ionic currents, calcium cycling, and contractility are similar to the more extensively studied hESC-CMs, but are still immature relative to adult hearts. Many factors can hinder the attainment of a well-defined myocyte phenotype, including variability introduced from different culture conditions, cell sources, differentiation times, and the retention of somatic epigenetic memory. Thus far, the cardiomyocytes that have been generated from hiPSCs have displayed an immature electrophysiological phenotype that is akin to fetal cardiac tissue. These cardiomyocytes display spontaneous automatic contractions, embryonic cardiac gene expression profiles, and fetal-like ion channel expression and calcium cycling. It is unclear at this time if these developmentally immature phenotypes will pose a limitation for downstream drug testing applications. Furthermore, the generation of immature cardiomyocytes with automaticity will likely pose a challenge for ultimate application in regenerative medicine. It is also not clear if these cardiomyocytes will engraft robustly and seamlessly in a damaged adult myocardium environment with a vastly incongruent physiological status. Approaches are needed to maturate the fetal-like hiPSC-CMs, and the detailed electrophysiologic and contractile characterization reviewed herein will be vital in solving this critical caveat.

The cardiac genetic disease models tested so far seem promising for mechanistic studies and future drug discovery, but it remains to be seen whether the level of developmental maturity and suppression of somatic epigenetic memory of hiPSC-CMs is sufficient for the cells to recapitulate all of the critical disease characteristics and to respond to pharmacological agents in a manner reflective of the genetic traits and function of cardiomyocytes in the patient population.

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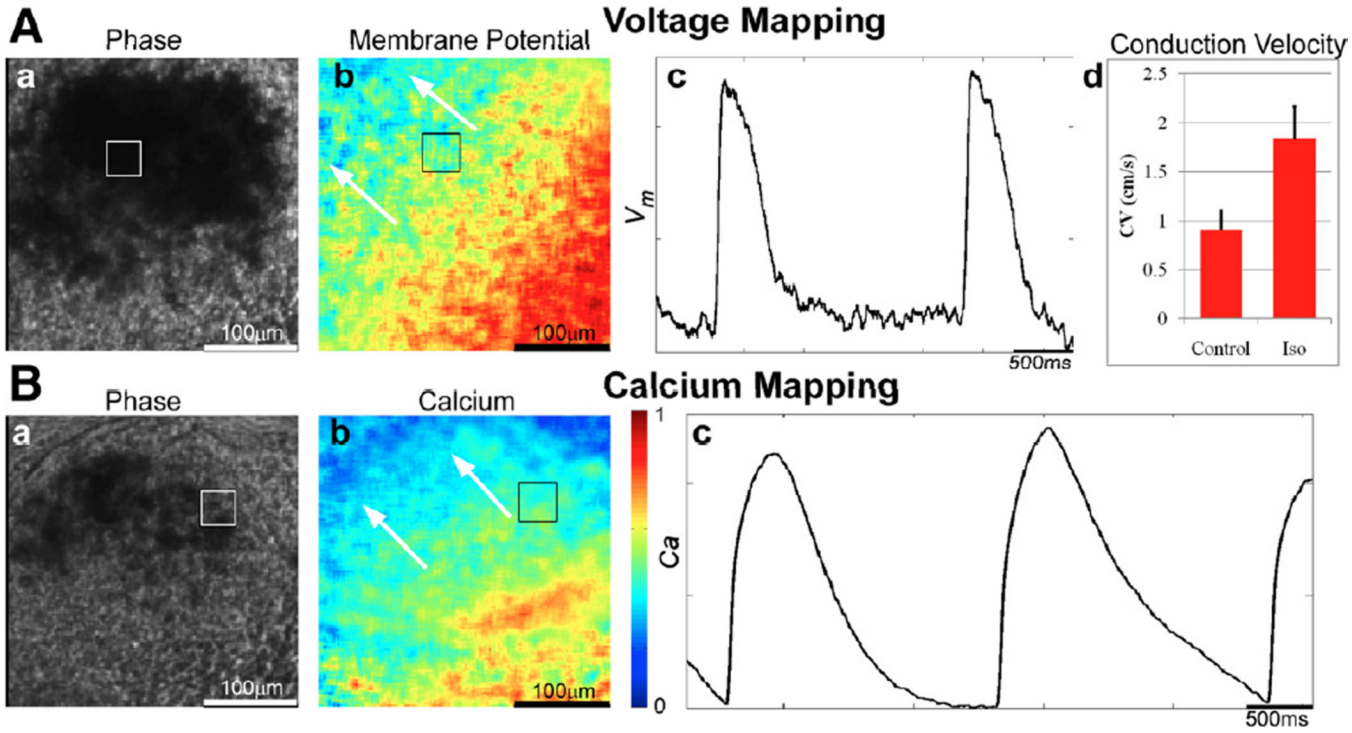

Fig. 1.

Voltage and calcium optical mapping of cord blood-derived hiPSC-EBs. (A) Voltage mapping. a. Phase image of CBiPSC6.2 EB at 4× magnification. b. Voltage map. hEB was stained with di-4-ANEPPS (voltage-sensitive fluorescent dye), and electrically field-stimulated (arrows indicate direction of electrical wave propagating across EB). c. Action potentials during 0.5 Hz field stimulation. d. Average conduction velocity (CV) measurements (mean ± standard deviation) for control and 15 min after addition of 50 μM isoproterenol during 0.5 Hz field stimulation. (B) Calcium mapping. a. Phase image of beating EB at 4× magnification. b. Calcium map (arrows indicate direction of calcium wave propagating across beating EB). c. Representative calcium transient (Ca) waveforms during 0.5-Hz field stimulation. In both A and B, the white square in a and b denote site of recording in c. Adapted from (Burridge et al., 2011).

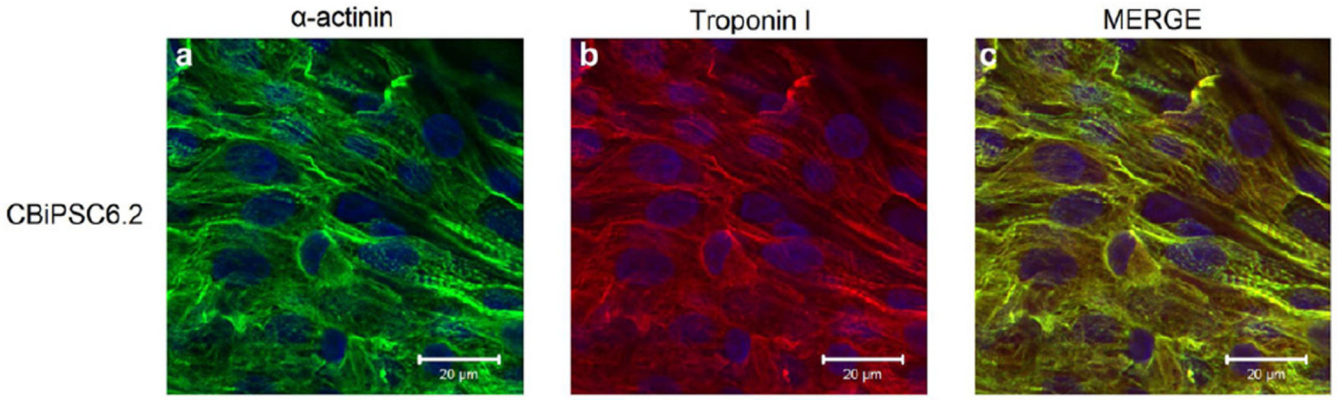

Fig. 2.
Immunocytochemistry for cardiac markers in hEB differentiated from CBiPSC6.2 cell line. Adapted from (Burridge et al., 2011).

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Table 1

Proportion of principal cellular electrophysiological phenotypes in hiPSC-CM cultures

N	Cell Line(s) (all from fibroblasts)	% Nodal-like	% Atrial-like	% Vent-like	Quantitative criteria[a]	Reference
23	fetal IMR90 C4	13	13	74	No	(Zhang et al., 2009)
20	newborn foreskin C1	20	10	70	No	(Zhang et al., 2009)
29	fetal IMR90 and adult dermal KS1	45	17	38	No	(Lee et al., 2011)
59	reprogrammed human fibroblast line	22	24	54	Yes	(Ma et al., 2011)

Notes:

[a] Quantitative criteria are those using numerical values of AP parameters to distinguish different phenotypes.

Table 2

Maturation of hiPSC-CM ion current gene expression

Current	Cell Line(s) (all from fibroblasts)	Gene	Gene product	Result	Reference
I_{Na}	dermal 201B7	*SCN5A*	$Na_v1.5$	Expressed in differentiated hiPSC-CMs but not undifferentiated cells	(Tanaka et al., 2009)
I_{CaL}	dermal 201B7	*CACNA1C*	$Ca_v1.2$	Expressed in differentiated hiPSC-CMs but not undifferentiated cells	(Tanaka et al., 2009)
	fetal IMR90			Increased approximately 2-fold in response to cyclic stress	(Tulloch et al., 2011)
	hFib2			Significantly upregulated over first 21 days of differentiation	(Zwi et al., 2009)
	foreskin	*CACNA1D*	$Ca_v1.3$	Significantly upregulated over first 21 days of differentiation	(Mehta et al., 2011)
	hFib2				(Zwi et al., 2009)
I_{Ks}	hFib2	*KCNQ1*	$K_v7.1$	Expressed in differentiated hiPSC-CMs but not undifferentiated cells	(Zwi et al., 2009)
I_{Kr}	dermal 201B7	*KCNH2 (HERG)*	$K_v11.1$	Expressed in differentiated hiPSC-CMs but not undifferentiated cells	(Tanaka et al., 2009)
	foreskin			Significantly upregulated over first 21 days of differentiation	(Mehta et al., 2011)
	hFib2				(Zwi et al., 2009)
I_f	foreskin	*HCN2*		Significantly upregulated over first 21 days of differentiation	(Mehta et al., 2011)
	hFib2				(Zwi et al., 2009)
I_{to1}	dermal 201B7	*KCND3*	$K_v4.3$	Present but no maturation effects were reported.	(Yokoo et al., 2009)
I_{K1}	dermal 201B7	*KCNJ2*	Kir2.1	Present but no maturation effects were reported.	(Yokoo et al., 2009)

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Table 3

Expression of calcium handling protein or gene products in hiPSC-CMs

Protein or Gene Product	Cell Line(s) (all from fibroblasts)	Methods	Level[a]	Result	Reference
NCX1	adult dermal KS1, fetal IMR90	RT-PCR	Single	Expressed in both hiPSC- and hESC-CMs , lower expression in hiPSC-CMs	(Lee et al., 2011)
	dermal, CPVT clones	PCR	EB	Expressed	(Novak et al., 2011)
SERCA2A	adult dermal KS1, fetal IMR90	RT-PCR	Single	Expressed in both hiPSC- and hESC-CMs (H7, HES3), lower expression in hiPSC-CMs	(Lee et al., 2011)
	dermal hIH-1	RT-PCR	Single	Expressed	(Itzhaki et al., 2011b)
RyR2	adult dermal KS1, fetal IMR90	RT-PCR	Single	Expressed in both hiPSC- and hESC-CMs (H7, HES3), lower expression in hiPSC-CMs	(Lee et al., 2011)
	dermal hIH-1	RT-PCR/Immunocyto chemistry	Single	Expressed	(Itzhaki et al., 2011b))
	foreskin C1, C2, C3	Immunocyto chemistry	EB	Expressed in both hiPSC- and hESC-CMs (H9.2)	(Germanguz et al., 2011)
CSQ2	adult dermal KS1, fetal IMR90	RT-PCR	Single	Expressed in both hiPSC- and hESC-CMs (H7, HES3), similar expression levels	(Lee et al., 2011)
	dermal hIH-1	RT-PCR	Single	Expressed	(Itzhaki et al., 2011b)
	foreskin C1, C2, C3	Immunocyto chemistry	EB	Expressed in both hiPSC- and hESC-CMs (H9.2)	(Germanguz et al., 2011)
$Ca_v1.2$	fetal 201B7	Unspecified	EB	Expressed	(Tanaka et al., 2009)
	hFib2	Unspecified	EB	Expressed	(Zwi et al., 2009)
IP3R2	dermal hIH-1	RT-PCR/ Immunocyto chemistry	Single	Expressed	(Itzhaki et al., 2011b)
PLN	fetal IMR90 C1, C4; foreskin C1, C2	RT-PCR	EB	Expressed in both hiPSC and hESC-CMs.	(Zhang et al., 2009)
	dermal hIH-1	RT-PCR	Single	Expressed	(Itzhaki et al., 2011b)

Notes:

[a] Level indicates whether contractility studies were performed in isolated cells (indicated by ‘Single’) or hEBs.

Table 4

SR calcium release and uptake in hiPSC-CMs

Property	Cell Line(s) (all from fibroblasts)	Level[a]	Result	Reference
SR Ca uptake	adult dermal KS1, fetal IMR90	Single	Slower Ca^{2+} transient decay in hiPSC-CMs vs hESC-CMs (H7, HES3)	(Lee et al., 2011)
	dermal hIH-1	Single	SERCA inhibitor eliminated Ca^{2+} transient	(Itzhaki et al., 2011b)
SR Ca release	newborn foreskin C1	EB	Caffeine-sensitive Ca^{2+} transient, similar response as hESC-CMs (HES2)	(Gupta et al., 2010)
	adult dermal KS1, fetal IMR90	Single	Caffeine- and RyRsensitive Ca^{2+} transient, smaller response in hiPSC-CMs vs hESCCMs (H7, HES3)	(Lee et al., 2011)
	dermal hIH-1	Single	Caffeine- and RyRsensitive Ca^{2+} transient	(Itzhaki et al., 2011b)
	foreskin C1, C2, C3	EB	contraction in hiPSCCMs, no response in hESC-CMs (H9.2)	(Germanguz et al., 2011)

Notes:

[a] Level indicates whether contractility studies were performed in isolated cells (indicated by ‘Single’) or hEBs.