Screening Drug-Induced Arrhythmia Events Using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes and Low-Impedance Microelectrode Arrays

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Cardiac toxicity is a major cause of drug attrition during preclinical development. In addition, the risk of drug-induced arrhythmia is the most common cause of restriction or withdrawal of drugs from the market. Between 1990 and 2001, 8 noncardiovascular drugs were withdrawn at an estimated cost of $12 billion as a result of problems such as delayed cardiac repolarization, prolonged QT interval, and Torsade de Pointes (TdP). TdP is a rare polymorphic ventricular tachyarrhythmia with a characteristic twist of the QRS complex around the isoelectric baseline that often resolves spontaneously but can also result in true ventricular tachycardia, ventricular fibrillation, and sudden cardiac death.

**Background**—Drug-induced arrhythmia is one of the most common causes of drug development failure and withdrawal from market. This study tested whether human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) combined with a low-impedance microelectrode array (MEA) system could improve on industry-standard preclinical cardiotoxicity screening methods, identify the effects of well-characterized drugs, and elucidate underlying risk factors for drug-induced arrhythmia. hiPSC-CMs may be advantageous over immortalized cell lines because they possess similar functional characteristics as primary human cardiomyocytes and can be generated in unlimited quantities.

**Methods and Results**—Pharmacological responses of beating embryoid bodies exposed to a comprehensive panel of drugs at 65 to 95 days postinduction were determined. Responses of hiPSC-CMs to drugs were qualitatively and quantitatively consistent with the reported drug effects in literature. Torsadogenic hERG blockers, such as sotalol and quinidine, produced statistically and physiologically significant effects, consistent with patch-clamp studies, on human embryonic stem cell–derived cardiomyocytes hESC-CMs. False-negative and false-positive hERG blockers were identified accurately. Consistent with published studies using animal models, early afterdepolarizations and ectopic beats were observed in 33% and 40% of embryoid bodies treated with sotalol and quinidine, respectively, compared with negligible early afterdepolarizations and ectopic beats in untreated controls.

**Conclusions**—We found that drug-induced arrhythmias can be recapitulated in hiPSC-CMs and documented with low impedance MEA. Our data indicate that the MEA/hiPSC-CM assay is a sensitive, robust, and efficient platform for testing drug effectiveness and for arrhythmia screening. This system may hold great potential for reducing drug development costs and may provide significant advantages over current industry standard assays that use immortalized cell lines or animal models. (Circulation. 2013;128[suppl 1]:S3-S13.)

**Key Words:** arrhythmias, cardiac genomics myocytes, cardiac pharmacogenetics pharmacology stem cells


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prolongation, early afterdepolarizations (EADs), ectopic beats, and increased spatial dispersion of repolarization are risk factors associated with TdP. EADs are considered the underlying mechanism that generates TdP because they can progress to triggered activity, lead to ectopic beats, and contribute to the dispersion of ventricular repolarization. Importantly, drugs that do not induce EADs, ectopic beats, or increased dispersion of ventricular repolarization have a low incidence of TdP.1.7,8

One of the problems in drug development is that primary human cardiomyocytes are difficult to obtain and survive only transiently in culture. The combination of stably transfected cell lines and automated, high-throughput patch clamping is a standard assay for cardiac safety screening because the majority of clinical cases of drug-induced cardiotoxicity have been associated with blockage of the hKv11.1 potassium channel, also known as hERG.3–10 The limitations of this approach are well understood because the QT interval is the net result of the activities of several interdependent ion channels.11 To address this issue, animal models, such as rabbit or dog Purkinje fibers, are used in later stages of the drug screening process to study the action potential (AP) via conventional patch clamping. However, the lack of sensitivity of these assays to torsadogenic drugs is well known.12–14 Furthermore, the vulnerability of the cardiomyocyte calcium current to run down because of dialysis of the cytoplasm with conventional whole-cell patch clamping limits the duration of drug tests.15–18

Human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) have great potential in drug screening because they express human ion channels similar to primary human CMs.19,20 As undifferentiated hiPSCs can, in theory, be cultured indefinitely, the supply of human CMs is likewise unlimited.21–23 Significantly, patient-specific hiPSC-CMs can be produced to study drug effects in the context of a particular risk factor or disease mutation.24–30 Microelectrode arrays (MEAs) have been used previously in many pharmacological studies using cardiac tissue, including hiPSC-CMs.26,28,31 MEAs can easily extract basic electrophysiological parameters, such as beat frequency and field potential duration (FPD), that correlate closely with cardiomyocyte AP durations (APD) obtained via patch clamping, as well as with heart rates and QT intervals obtained via clinical ECGs. Furthermore, the noninvasive nature of MEAs allows the study of drug effects on beating rate and rhythm. Although many academic laboratories, pharmaceutical companies, and contract research organizations have begun to adopt MEA assays for hiPSC-CM research, no published study has compared results from this approach with clinical data to address whether the MEA/hiPSC-CM system has predictive value for drug effects on beating rate and QT intervals.

Here, we report and quantify drug-induced EADs, ectopic cardiomyocyte contraction, and the pharmacology of a comprehensive panel of drugs from various classes using an MEA/hiPSC-CM platform. Our study demonstrates a novel means of studying cardiomyocyte pharmacology and toxicology by using the MEA/hiPSC-CM technology to evaluate >10 known arrhythmia-regulating compounds through a broad set of cellular, genetic, and physiological assays.

Methods

An expanded Methods section is available in the online-only Data Supplement.

Derivation of hiPSC-CMs From Embryoid Bodies

Colonies fulfilling established stemness criteria were differentiated into cardiomyocytes using standard 3-dimensional differentiation protocols and maintained in a 5% CO2/air environment. Briefly, on day 0 hiPSC colonies were dissociated with Accutase (Sigma) into small clumps of 10 to 20 cells. Cells were resuspended in 2 mL of basic media containing StemPro34 (Invitrogen), 2 mM/L glutamine (Invitrogen), 0.4 mM/L monothioglycerol (Sigma), 50 μg/mL ascorbic acid (Sigma), and 0.5 ng/mL bone morphogenic protein 4 (BMP4) (R&D Systems, Minneapolis, MN) to form embryoid bodies (EBs). For the first 1 to 4 days, cells were treated with 10 ng/mL BMP4, 5 ng/mL human basic fibroblast growth factor (bFGF) (R&D Systems), and 3 ng/mL activin A (R&D Systems) in the basic media. On days 4 to 8, EBs were refed with basic media containing human 50 ng/mL Dickkoff related protein 1 (R&D Systems) and 10 ng/mL human vascular endothelial growth factor (R&D Systems). After day 8, cells were cultured in basic media containing 5 ng/mL human bFGF and 10 ng/mL human vascular endothelial growth factor. To further corroborate data obtained from EB differentiation, hiPSC-CMs were also created through a monolayer differentiation technique at 30 to 45 days post-induction for consistency with commercially available hiPSC-CMs (Methods in the online-only Data Supplement).

Gene Expression and Immunocytochemistry

RNA was extracted with the miRNeasy kit (Qiagen). cDNA was synthesized using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA), and real-time polymerase chain reaction was performed with Taqman assays (Applied Biosystems) using StepOne (Applied Biosystems). The Hierarchical clustering was performed and the heatmap was generated by TM4 (www.tm4.org). Immunostaining was performed according to previously established protocols. Imaging was performed using a DMLM-LED inverted tissue culture microscope (Leica Microsystems, Buffalo Grove, IL) or an A1-R Resonant Confocal System running NIS Elements software (Nikon, Tokyo, Japan).

Microelectrode Array Recordings

Beating EBs were plated on 0.1% to 0.2% gelatin-coated AL-MED P0004A MEA probes (Alpha Med Scientific, Osaka, Japan; Figure 1A in the online-only Data Supplement). Pharmacological responses were examined 65 to 95 days post-induction in EBs and 30 to 45 days post-induction in monolayers. Before MEA recordings, audio video interactive videos were recorded on the DMIL-LED microscope. Signals were acquired at 20 kHz using a MED64 amplifier (Alpha Med Scientific) and digitized via an Optiplex 390 PC (Dell, Round Rock, TX) with PCI-6071 A/D cards (National Instruments, Austin, TX) running Mobius QT software (Witwrex, Inc, Tustin CA; Figure IB in the online-only Data Supplement). Data were analyzed by Mobius QT software (Witwrex, Inc, Tustin CA), Igor Pro (Wave Metrics), Microcal Origin 6.1 (OriginLab), or Prism 5 (GraphPad). Steady state parameters were averaged, and FPD was normalized to beat rate using the Bazett correction formula (corrected FPD = [FDP/√(R)], where FPD is the field potential duration and R is the heart rate).
Statistical Methods
Data are presented as mean±SEM. All statistical analyses were performed using the software SigmaPlot and SigmaStat. In our study, before performing the Student t-test between 2 groups of data, we ran the Shapiro–Wilk test, and the data set with a P<0.05 was considered normally distributed. Afterward, we examined the 2 groups of data using the equal variance test, and the 2 groups of data with P>0.05 were considered to have similar variance. Only data that passed both the tests were further analyzed with Student test, and the 2-tailed P<0.05 were considered to be statistically significant between the 2 groups. Responses to drugs were normalized and compared with baseline via t tests, with significant differences defined by P<0.05. FPD increases of >10% were considered physiologically significant.

Results
Expression of Cardiomyocyte Markers
We confirmed the presence of genes specific to the cardiomyocyte lineage encoding a variety of protein classes. Cardiogenic structural genes encoding sarcomeric myofilament proteins included ACTC1 (α-actin 1), MYL2 (myosin regulatory light chain 2), MYH6 (myosin heavy chain 6, α), and MYH7 (myosin heavy chain 7, β; Figure II in the online-only Data Supplement). Importantly, the major ion channel genes essential for cardiomyocyte function were also expressed (Figure 1A). A complete list of all structural and functional genes evaluated in this study can be found in Table I in the online-only Data Supplement. Hierarchical clustering analysis demonstrates that our hiPSC-CMs were more similar to cells obtained from human adult ventricles than undifferentiated hiPSCs. Expression of sarcomeric proteins was confirmed via immunofluorescence and laser confocal microscopy. Cardiac troponin T staining of hiPSC-CMs showed a typical striated pattern intercalated with α-actin along the sarcomeric Z lines (Figure 1B). hiPSC-CMs with organized parallel myofilaments and cylindrical morphology were often observed. Furthermore, hiPSC-CMs derived using the iPSC monolayer differentiation method exhibited staining profiles comparable with hiPSC-CMs derived using the EB method (Figure III in the online-only Data Supplement).

Characterization of Cardiomyocyte EBs
Video contractility analyses determined that hiPSC-CMs composed the vast majority of EBs (Movie I in the online-only Data Supplement). The average contractile EB area was 88.4±5.4% (n=4), consistent with the percentage of troponin T (+) cells (81.34±0.91%) in dissociated EBs (Figure 2). Ventricular-like, atrial-like, and nodal-like CM subtypes were identified via their AP parameters (Table 1 and Figure IV in the online-only Data Supplement). Cells with ventricular-like APs were the most frequently encountered, comprising 67% of all tested hiPSC-CMs, followed by atrial-like cells (28%) and nodal-like cells (5%).

Baseline Microelectrode Array Electrophysiology
Spontaneous beating and electric activity were present in all plated EBs as shown in Movies II to VI in the online-only Data Supplement. By comparison, spontaneous beating activity was also observed in hiPSC-CMs derived from iPSC monolayer differentiation (Movie VII in the online-only Data Supplement). Baseline electrophysiological parameters recorded on day 75 are presented in Table 2. Representative traces for the subject’s ECG (lead 4), hiPSC-CM’s AP, and beating EB’s FPD are shown in Figure 3. The average beating frequency was 35 bpm, and the mean FPDc was 473 ms, suggesting ventricular-like CMs were predominant in our EBs (n=5), consistent with gene expression clustering and single-cell patch-clamp data. The baseline is stable with little change in beating frequency and FPDc during a 10-minute period (Figure V in the online-only Data Supplement). Furthermore, hiPSC-CMs derived using the iPSC monolayer differentiation method also exhibited electrophysiological parameters analogous to EB-derived hiPSC-CMs (Figures VI and VII in the online-only Data Supplement).

Neurohormonal Responses
The autonomic nervous system plays an important role in the regulation of heart rate and contraction force. To assess the sensitivity of hiPSC-CMs to neurohormonal regulation, we administered the adrenergic catecholamine norepinephrine (NE), the mixed β-agonist isoproterenol (ISO), and the muscarinic cholinomimetic carbamyl choline. NE had a clear dose-dependent effect on the beat frequency of all EBs tested (Figure 4A and 4B). The onset of responses with both adrenergic drugs was quick, usually within 3 minutes (Figure 4C and Figure VIII A in the online-only Data Supplement). Although there was occasional beat-to-beat variability and usually a

Figure 1. Human induced pluripotent stem cell–derived cardiomyocyte (hiPSC-CM) expression of cardiac ion channel genes and sarcomeric proteins. A, Heat map showing the gene expression levels of the main adrenoreceptors and ion channels essential for cardiomyocyte function in hiPSCs and human adult heart chambers. Color scale represents delta-delta Ct values relative to the expression levels in hiPSC-CMs. Green indicates upregulation and red downregulation. Strong red corresponds to genes that are not expressed. Average linkage cluster for the samples is presented (n=4). B, Confocal microscopy image of α-actinin (top) and troponin T (middle) immunostaining demonstrates the presence of cardiac-specific sarcomeric proteins in a single hiPSC-CM. The inset in the merged image at the bottom shows organized horizontal myofilaments, with parallel red striations indicating the sarcomeric Z lines. DAPI indicates 4',6-diamidino-2-phenylindole; LA, left atrium; LV, left ventricle; RA, right atrium; and RV, right ventricle.
rebound from the maximal effect, the increases in beat frequency stabilized and persisted until the end of the 10-minute recording (Figure 4D and Figure VIIIB in the online-only Data Supplement). Interestingly, the same EBs tested 9 days earlier at 67 days postinduction with ISO had clear responses in only 75% of the EBs tested (n=4; Figure VIIIC and VIIID in the online-only Data Supplement), and only half of them demonstrated clear dose dependence with a half-maximal excitatory concentration (EC$_{50}$) of 72.03±16.80 nm/L (Figure IXA in the online-only Data Supplement), suggesting that the adrenoreceptor sensitivity of some of these EBs was immature. Notably, the EC$_{50}$ for the effect of ISO on heart rate is close to the EC 50 for the effect of ISO on adult human heart slice contractility of 118±20 nm/L.\(^3\) The half-maximal excitatory concentration (EC 50) for the effect of NE on heart rate was 41.66±16.50 nm/L (n=4; Figure 5A), although responses were often evident at 1 nmol/L for both ISO and NE (data not shown). ISO and NE also had a clear dose-dependent effect on FPD, shortening it consistently (Figure 5B and 5C and Figures IXB and IXC in the online-only Data Supplement).

Heart rate is an important variable affecting the QT interval, and correction formula have been used since 1920, with the Bazett formula being the most popular.\(^3\)\(^5\) Indeed, the Bazett correction produced a flat Hill fit to the NE frequency response (Figure 5D). Nevertheless, it is well known that the Bazett formula overcorrects at high beat frequencies, as our results with ISO illustrate (Figure IXD in the online-only Data Supplement).\(^1\) Carbachol (CCh) produced a dose-dependent decrease in heart rate, regardless of prior adrenergic stimulation (Figure XA–XD in the online-only Data Supplement), and consequently resulted in FPD prolongation (Figure XIA in the online-only Data Supplement). The CCh-induced decreases in heart rate with or without ISO administration were not significantly different at any concentration tested. On average, maximal decreases in heart rate exceeded 50% at 100 μmol/L, and one third of the EBs lost their spontaneous activity, regardless of prior adrenergic stimulation. CCh also increased FP amplitude in a dose-dependent manner (Figure XIB in the online-only Data Supplement).

### Responses to hERG Blockers

A variety of drugs can block the hERG channel because of its unique structural promiscuity.\(^3\)\(^7\)\(^8\) We tested the clinically relevant drugs sotalol and quinidine, which are currently prescribed for atrial fibrillation and ventricular tachycardia. First, we tested the mixed antiarrhythmic drug sotalol to gauge the time course of responses. The onset of the effect on the EB’s FPD was comparable with that reported for patch-clamped hESC-CMs.\(^1\) As shown in Figure 6A, the onset of the effect of sotalol at 30 μmol/L was almost immediate and typically stabilized within ≈7 minutes. As with ISO, the time course of

### Table 1. Baseline Electrophysiology in Single hiPSC-CMs Obtained via Patch Clamping

<table>
<thead>
<tr>
<th>CM Subtype</th>
<th>n (%)</th>
<th>bpm</th>
<th>MDP, mV</th>
<th>Overshoot, mV</th>
<th>APA, mV</th>
<th>APD$_{90}$, ms</th>
<th>V$_{max}$ (V/S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodal-like</td>
<td>2 (5)</td>
<td>72±10</td>
<td>−32.6±5.4</td>
<td>33.4±3.2</td>
<td>67.4±6.3</td>
<td>244.5±30.6</td>
<td>2.9±1.4</td>
</tr>
<tr>
<td>Atrial-like</td>
<td>11 (28)</td>
<td>56±12</td>
<td>−50.6±4.2</td>
<td>42.5±6.5</td>
<td>93.3±6.8</td>
<td>276.3±20.5</td>
<td>27.4±10.3</td>
</tr>
<tr>
<td>Ventricular-like</td>
<td>27 (67)</td>
<td>50±15</td>
<td>−52.6±6.3</td>
<td>45.5±4.1</td>
<td>98.7±10.4</td>
<td>342.7±28.7</td>
<td>26.8±14.2</td>
</tr>
</tbody>
</table>

Mean±SEM. APA indicates action potential amplitude; APD$_{90}$, action potential duration at 90%; and bpm, beats per minute; CM, cardiomyocyte; hiPSC-CM, human induced pluripotent stem cell-derived cardiomyocytes; MDP, maximal diastolic potential; and V$_{max}$, maximal upstroke velocity.
responses to sotalol was concentration dependent. For example, we observed the stabilization of the effect within 2 minutes with 100 μmol/L (data not shown). As expected, the increase in FPD was concentration dependent (Figure 6B). Sotalol also produced a dose-dependent decrease in beat frequency (Figure 6C), likely because of its activity as a β-adrenoreceptor blocker, making a correction procedure necessary. The average increase in FPDc because of sotalol was statistically and physiologically significant at 100 μmol/L and had a maximal prolongation of 36% at the highest tested sotalol concentration of 500 μmol/L (n=4), with a half-maximal effect at 214.1±102.0 μmol/L (Figure 6D). It is noted that 15 μM is the maximum ETPC, yet we tested up to an unphysiologic concentration of 500 μM in an unsuccessful attempt to determine the asymptote of the Hill function. A clear sotalol-induced, dose-dependent, FPDc prolongation was also obtained using age-matched monolayer-derived hiPSC-CMs (Figure XIIIA in the online-only Data Supplement). Consistent with APD studies on hESC-CMs, quinidine also induced FPDc prolongation that was statistically significant at 100 mmol/L and physiologically significant at 300 mmol/L, with a half-maximal effect on FPDc prolongation at 333.1 nmol/L (Figure XIIIIA and XIIIIB in the online-only Data Supplement).13 A dose-dependent decrease in FP amplitude and maximal upstroke velocity (V_{max}) was also observed, likely because of the well-known capability of quinidine to block the sodium current (Figure XIIIC and XIIID in the online-only Data Supplement). Most importantly, hERG blockers induced the arrhythmic activity that results in TdP. We observed the characteristic notched or bifurcated repolarizing waves described in patients with long QT syndrome-2 and the long QT syndrome-2 dog model or bifurcated repolarizing waves described in patients with long QT syndrome-2 and the long QT syndrome-2 dog model with 100 μmol/L sotalol (Figure 7A).29,40 At higher concentrations, we observed ectopic beats and the short-long-short rhythm known to precede TdP (Figure 7B).10,41,42 One third of our sample tested with sotalol (n=6) displayed the aforementioned arrhythmias (Figure XIIIB and XIIIC in the online-only Data Supplement). Quinidine also induced arrhythmic activity in 40% of the EBs tested (n=5), eliciting EADs and ectopic beats at concentrations as low as 1 μmol/L (Figure 7C and Figure XIVB in the online-only Data Supplement), well within the free effective therapeutic plasma concentration of 0.9 to 3.2 μmol/L.7 Interestingly, the noted waveform morphology seen with sotalol is similar to that seen in quinidine-induced EADs. Finally, we also observed sotalol-induced EADs at a higher dose (100 μmol/L) when using monolayer-derived hiPSC-CMs (n=3; Figure XV in the online-only Data Supplement). Untreated control EBs did not demonstrate EADs or ectopic beats (data not shown).

### Responses to a False-Positive hERG Blocker

Verapamil is a phenylalkylamine L-type calcium channel blocker that also has a potent effect on hERG. Blocking of L-type calcium channel leads to QT shortening, whereas blocking of hERG results in QT prolongation. Given the dual roles to shape the QT, however, verapamil is a well-known false-positive hERG blocker with no reports of QT prolongation orTdP in humans.7 In our hiPSC-CM sample, verapamil induced progressive FPD shortening with increasing concentrations (Figure 8A). The half-maximal effect on FPDc occurred at a concentration of 169±24 nmol/L (n=4; Figure 8B), reasonably close to the free effective therapeutic plasma concentration of 25 to 81 nmol/L.7 Interestingly, half of our sample’s spontaneous electric activity was abolished at 1 μmol/L and all our EBs stopped beating at 10 μmol/L (Figure XVA in the online-only Data Supplement). Spontaneous beating was 4× more sensitive to verapamil at earlier developmental stages (half-maximal inhibitory concentration, or IC_{50}, at 82 days postinduction 410.65±40.80 nmol/L versus 103.20±6.03 in 30-day-old EBs), Verapamil also decreased the amplitude of initial fast depolarizing spike in a dose-dependent manner (Figure XVIB in the online-only Data Supplement).

### Responses to a False-Negative hERG Blocker

Alfuzosin is a selective α-adrenoreceptor blocker approved by the Food and Drug Administration for the treatment of benign prostatic hyperplasia. Alfuzosin is a rare drug that delays cardiac repolarization by increasing sodium current.43 This mechanism of QT prolongation is similar to the hereditary arrhythmia, long QT syndrome-3. Alfuzosin weakly inhibits hERG current tested in Chinese hamster ovary or human embryonic kidney 293 cells stably overexpressing hERG,

### Table 2. Baseline Electrophysiology in hiPSC-CM Embryoid Bodies Obtained via MEAs

<table>
<thead>
<tr>
<th>hiPSC-CM EBs</th>
<th>bpm</th>
<th>FPD (ms)</th>
<th>FPDc (ms)</th>
<th>V_{max} (mW/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=5</td>
<td></td>
<td>34.95±4.54</td>
<td>629.87±64.58</td>
<td>472.53±58.99</td>
</tr>
</tbody>
</table>

Mean±SEM. Bpm indicates beats per minute; FPD, field potential duration; FPDc, corrected field potential duration; hiPSC-CM EBs, human induced pluripotent stem cell–derived cardiomyocyte embryoid bodies; MEA, microelectrode array; and V_{max}, maximal upstroke velocity.

### Figure 3. QT interval, action potential duration, and field potential duration.

A, ECG (lead 4) obtained from the subject illustrating the QT interval. B, Action potential recorded via whole-cell patch clamping of a representative ventricular human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) from a dissociated embryoid body (EB) showing the action potential durations (APD). C, Field potentials recorded from a representative EB via microelectrode arrays denoting the electrophysiological parameters of interest. Note the similarity between the ECG inset on the top left corresponding to the time course of a typical action potential recorded via conventional patch clamping on the top right inset.
hence the readout is safe using the hERG blockade assay. However, it can delay cardiac repolarization (QT prolongation) by increasing the sodium current, thus leading to false-negative readout. Using our MEA/hiPSC-CM platform, we were able to record dose-dependent increases in FPD (Figure 8C). Increases in FPDc were statistically and physiologically significant at the clinically relevant concentration of 300 nmol/L (Figure 8D; n=4), consistent with previous studies.43 The average FPDc had a maximal prolongation of 65% at 1 μmol/L, and spontaneous electric activity was abolished in half of our sample at this concentration. The largest increase in FPDc was 220% at 10 μmol/L. Interestingly, we did not see any EADs, but this is consistent with the rabbit purkinje fiber (PF) and Langendorff-perfused heart.43

Responses to Other Ion Channel Blockers

To further characterize the pharmacology of our hiPSC-CMs, we investigated the sensitivity to other ion channel blockers. First, we tested nifedipine, a potent dihydropyridine L-type calcium channel blocker. A concentration-dependent decrease in FPD was observed, consistent with the effects seen with verapamil, and was both physiologically and statistically significant at concentrations of ≥10 nmol/L (Figure XVIIA and XVIB in the online-only Data Supplement). This is also consistent with the effect on hESC-CM APD.13 Nifedipine produced an important reduction in FP amplitude with increasing dosage. Beat frequency was also sensitive to nifedipine, consistent with the effect of verapamil. The loss of spontaneous activity was observed in 75% of our sample at 100 nmol/L, and at 300 nmol/L all our EBs stopped beating, similar to previous reports.1 The IC50 for FPDc shortening was 41.96±4.62 nmol/L, consistent with the IC50 for Ca current block in patch-clamped hiPSC-CMs and reasonably close to the reported free effective therapeutic plasma concentration of 3.1 to 7.7 nmol/L.7,21 For nifedipine treatment, similar...
dose-dependent FPDc shortening was obtained using age-matched hiPSC-CMs derived from the monolayer method (Figure XVII in the online-only Data Supplement). We then tested lidocaine, an anesthetic that blocks fast voltage-gated sodium channels. Like quinidine, a dramatic dose-dependent decrease in the amplitude of the fast depolarizing spike of the field potential and $V_{\text{max}}$ was observed (Figure XVIC and XVID in the online-only Data Supplement). The reduction in $V_{\text{max}}$ was statistically significant at 10 $\mu$mol/L, consistent with the effect on hESC-CM APDs. In addition, half of the sample stopped beating at 300 $\mu$mol/L, the maximal concentration tested, in line with prior studies.

**Discussion**

The MEA/hiPSC-CM assay addresses the unmet need in the pharmaceutical industry for a humanized cardiac safety platform with better predictive power. Species differences and the lack of the complex channel interactions in hERG-transfected cell lines limit the sensitivity and specificity of the standard methodologies for cardiac toxicity-drug safety screening. hiPSC-CMs express virtually all known ion channels and subunits consistent with adult human ventricular tissues, as well as APs consistent with ventricular myocyte electrophysiology, despite the immature morphology and spontaneous beating of hiPSC-CMs. Furthermore, this spontaneous activity can be exploited to investigate chronotropic drug effects, an advantage of using a noninvasive system, such as the MEA platform. In addition, our multicellular preparation reduces the vulnerability of the reductionist single-cell preparation and the intrinsic differences between cells. In fact, close examination of our dose–response studies reveals small variability in the responses at baseline and initial doses.
Furthermore, we did not observe EADs, ectopic beats, or any other arrhythmic activity in baseline studies.

Our findings have several important implications for the use of MEA/hiPSC-CM assay to detect drug-induced arrhythmias. This is the first quantitative characterization of the pharmacology and ability of hiPSC-CMs to detect drug-induced arrhythmias using MEAs and a comprehensive panel of drugs from various classes. We have shown that responses of hiPSC-CMs to neurohormonal drugs and ion channel blockers are qualitatively and quantitatively consistent with previous reports, including APD and FPD measures on hESC-CMs. These findings correlate with the free ETPC in humans, confirming their suitability for testing drug effectiveness. We also show that the MEA/hiPSC-CM assay is sensitive to ion channel blockers affecting specific phases of the cardiac AP: (1) sodium channel blockers lidocaine and quinidine, which slow $V_{\text{max}}$ and reduce the FP amplitude in phase 0; (2) L-type calcium channel blockers nifedipine and verapamil, which shorten the AP plateau (phase 2); and (3) class III antiarrhythmic hERG blockers, which delay phase 3 repolarization. Furthermore, we have shown that the MEA/hiPSC-CM platform can clearly identify false-negative and false-positive torsadogenic drugs. Nonetheless, characterization of Kv4.3, KCNQ1, and Kir2.1, including their conductances and modulators, is necessary. For example, some studies suggest that underexpression of Kir2.1 (and thus the $I_{\text{K1}}$ current) causes spontaneous beating in hESC-CMs and hiPSC-CMs. As mentioned previously, this spontaneous activity can be exploited to investigate chronotropic drug effects; for example, we found that calcium currents have a pivotal role in the generation of spontaneous electric activity in hiPSC-CMs. Notably, we recorded significant developmental changes in the sensitivity to verapamil, consistent with the developmental upregulation of ion channel expression that others have described. The determination of the appropriate time points to test drug efficacy and safety research on hiPSC-CMs is imperative. We believe that hiPSC-CMs will eventually allow us to conduct personalized drug screening assays because these stem cell–derived cardiomyocytes have the same genomic background and express the same genetic mutations as the patient.

Arrhythmia detection using impedance measures and hiPSC-CMs is already being used by pharmaceutical companies and contract research organizations. Here, we present a refinement on these methodologies and show that the low-impedance MEA/hiPSC-CM platform can be used to identify and quantify arrhythmic events such as EADs and ectopic beats, the actual underlying mechanisms of TdP, confirming the presence of the phase 2 to 3 window necessary for inward current to produce these arrhythmic events. Importantly, the incidence of drug-induced arrhythmias was consistent with animal models, especially the dog. The incidence of sotalol-induced arrhythmias at a concentration of 100 μmol/L was close to that of the dog left ventricular wedge and dog mid-myocardial cardiomyocyte(Figure XIV A in the online-only Data Supplement). The incidence of quinidine-induced arrhythmias at a concentration of 1 μmol/L was similar to that of the dog Purkinje fiber(Figure XIVB in the online-only Data Supplement). Only 2 previous studies evaluated arrhythmia quantification, but these were on single hESC-CMs using patch-clamp or sharp electrodes. Unfortunately, arrhythmic behavior is often encountered in both normal hESC-CMs and hiPSC-CMs. The use of EADs or ectopic beats as an end point for drug safety has a greater prognostic value than hERG IC_{50} assays and could potentially reduce the attrition of drugs with therapeutic potential. In fact, in a thorough review of the literature, Redfern et al. found that the hERG IC_{50} assay could only predict safety if the block occurred at a 30-fold concentration above the effective therapeutic plasma concentrations. At present, patch clamping is the gold standard for assessing whether a drug affects a specific ion channel, such as hERG. However, because many drugs have pleiotropic effects, the MEA/hiPSC-CM assay is more likely to identify arrhythmogenic effects in human cardiac myocytes that go beyond simple hERG effects alone, as our results with alfuzosin demonstrate (Figure 5A). The MEA/hiPSC-CM assay, thus, may be a better

![Graph A](image1.png)
![Graph B](image2.png)
![Graph C](image3.png)
![Graph D](image4.png)

**Figure 8.** Microelectrode array/human induced pluripotent stem cell–derived cardiomyocyte (MEA/hiPSC-CM) platform identifies false-positive and false-negative hERG blockers. A. Dose-dependent shortening of field potentials caused by verapamil in a representative embryoid body (EB). Arrow points to the left-shifting peak of the repolarizing wave to indicate shortening of the field potential duration (FPD). B. The dose-dependent decreases in the normalized mean corrected FPD (FPDc) could be fitted nicely with the Hill equation (red line), evidencing a half-maximal effect at 169.28±24.00 nmol/L (n=4). C. Alfuzosin induced dose-dependent FP prolongation in our hiPSC-CMs as shown in this graph of a representative EB’s responses. Arrow points to the right-shifting peak of the repolarizing wave to indicate lengthening of the FPD. D. FP prolongation was statistically and physiologically significant at ≥300 nmol/L, as noted by the asterisks (n=4).
first-line assay for determining cardiotoxicity that could complement patch-clamp studies to pinpoint the specific channels underlying the QT prolongation or arrhythmic behavior.

Our findings further indicate that the MEA/hiPSC-CM assay may also be more cost-effective and efficient than either PF assays or patch-clamping single hiPSC-CMs. As shown in Figure 5A, the latency for sotalol prolongation is much shorter than the PF assay and comparable with patch-clamp assays.13 As continuous perfusion is avoided to prevent mechanical stimulation that may affect beating frequency, there is approximately a 35-fold reduction in test article consumption compared with the PF assay and a 5-fold reduction compared with the patch-clamp assay. In addition, perforated patch-clamping techniques require 15 to 20 minutes to achieve a gigaseal and then a stable membrane resistance in addition to the drug exposure time. MEAs only require culturing the sample on the probe. This increases the productivity significantly because data are always obtained with MEAs.

Although patient-specific hiPSC-CMs hold tremendous potential in terms of their applications for personalized drug screening, there are several limitations at present. A major issue associated with iPSC-derived cells is their immaturity compared with primary cells obtained from human adult tissues. Several landmark in vitro disease modeling studies have shown that neurons, hepatocytes, endothelial cells, and cardiomyocytes derived from iPSCs are developmentally immature and do not perfectly replicate the physiological properties of their natural adult counterparts.59-62 Previous work has shown that hiPSC-CMs exhibit functional but incomplete calcium handling components in the sarcoplasmic reticulum, leading to an electrophysiological output that imperfectly replicates that of adult cardiomyocytes.60 Both hESC-CMs and hiPSC-CMs could be better characterized as resembling human fetal cardiomyocytes as opposed to adult cardiomyocytes in terms of their electrophysiology and gene expression.63-65 It is also important to note that whereas immature hiPSC-CMs can develop specific disease phenotypes after 2 months of in vitro culture, a patient’s clinical phenotype can take years or decades to manifest. Finally, it is critical to investigate means of improving hiPSC-CM yield, cell homogeneity, and maturity during the in vitro differentiation process if these cells are to be used for large-scale drug testing studies. In addition to the aforementioned method of differentiating EBs to iPSC-CMs, our group and others have started using a monolayer-based differentiation approach to obtain >80% yields of functioning hiPSC-CMs.66 Differentiation protocols will improve as we devise more effective methods of fine-tuning cardiac fate specification in vitro. Despite the current limitations associated with hiPSC-CM technology, we believe that even fetal-like hiPSC-CMs hold a distinct edge over cells from transgenic animals when it comes to pharmacological drug screening assays for the simple fact that hiPSC-CMs are derived from human tissues and are being used to study human diseases.

In summary, we have recapitulated drug-induced arrhythmias with MEA on hiPSC-CMs. Our results support the use of the MEA/hiPSC-CM platform for drug development. These hiPSC-CMs represent a useful tool for safety pharmacology that can complement the current industry standards and have demonstrated potential for personalized therapeutics. We foresee the implementation of these methodologies in future drug safety practices and guidelines.

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Disclosures

Drs Wu, Robbins, and Lee hold founding shares in Stem Cell Theranostics, an iPSC drug screening company. Dr Navarrete consults for Alpha MED Scientific.

References


