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Original Article

Human induced pluripotent stem cell-cardiomyocytes for cardiotoxicity assessment: a comparative study of arrhythmia-inducing drugs with multi-electrode array analysis

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ABSTRACT Reliable preclinical models for assessing drug-induced cardiotoxicity are essential to reduce the high rate of drug withdrawals during development. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as a promising platform for such assessments due to their expression of cardiac-specific ion channels and electrophysiological properties. In this study, we investigated the effects of eight arrhythmogenic drugs—E4031, nifedipine, mexiletine, JNJ303, flecainide, moxifloxacin, quinidine, and ranolazine—on hiPSC-CMs derived from both healthy individuals and a long QT syndrome (LQTS) patient using multi-electrode array systems. The results demonstrated dose-dependent changes in field potential duration and arrhythmogenic risk, with LQTS-derived hiPSC-CMs showing increased sensitivity to hERG channel blockers such as E4031. Furthermore, the study highlights the potential of hiPSC-CMs to model disease-specific cardiac responses, providing insights into genetic predispositions and personalized drug responses. Despite challenges related to the immaturity of hiPSC-CMs, their ability to recapitulate human cardiac electrophysiology makes them a valuable tool for preclinical cardiotoxicity assessments. This study underscores the utility of integrating patient-derived hiPSC-CMs with advanced analytical platforms, such as multi-electrode array systems, to evaluate drug-induced electrophysiological changes. These findings reinforce the role of hiPSC-CMs in drug development, facilitating safer and more efficient screening methods while supporting precision medicine applications.

INTRODUCTION

The need for reliable methods to predict drug-induced cardiotoxicity has become more urgent as cardiovascular toxicity remains a leading cause for withdrawing a drug from clinical development [1,2]. Current preclinical models, such as animal testing and non-cardiac cell lines, present significant limitations due to species-specific differences and lack of human cardio-specific properties [3]. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have emerged as a critical model for investigating human cardiac physiology, owing to their ability to closely mimic the genetic and physiological characteristics of human cardiomyocytes [4]. Unlike conventional models such as HEK cells, which rely on artificial overexpression of ion

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channels, hiPSC-CMs express a comprehensive array of cardiac ion channels and proteins, providing a more physiologically relevant platform for evaluating drug effects on human cardiac cells [5]. Despite concerns regarding the immaturity of hiPSC-CMs compared to adult cardiomyocytes — particularly in ion channel expression and contractile function — hiPSC-CMs are still considered more appropriate for cardiotoxicity assessments than non-cardiac cell lines [3,5]. The expression of cardiac-specific ion channels and contractile activity makes hiPSC-CMs a valuable tool for assessing drug-induced cardiotoxicity.

Multi-electrode array (MEA) systems enable real-time, non-invasive measurement of cellular electrical activity and are effective for evaluating cardiotoxicity and electrophysiological drug responses [5]. Validated assessments, such as the Comprehensive *in Vitro* Proarrhythmia Assay (CiPA), have facilitated the use of hiPSC-CMs to evaluate cardiac safety during drug development [3]. Moreover, the ability to generate patient-specific hiPSC-CMs opens new possibilities for personalized medicine, allowing drug responses to be customized to individual genetic profiles, thereby enhancing therapeutic safety and efficacy [4,6].

In this study, we evaluated the electrophysiological effects of eight arrhythmogenic drugs (E4031, nifedipine, mexiletine, JNJ303, flecainide, moxifloxacin, quinidine, and ranolazine) on hiPSC-CMs using MEA systems. MEA technology offers a non-invasive, label-free platform to measure field potentials and detect drug-induced changes in cardiac repolarization and proarrhythmic risks [5]. We employed three healthy control cell lines (CMC-006, CMC-011, CMC-016) and one cell line (DPHC01i-AR) derived from a patient with long QT syndrome (LQTS) to assess concentration-dependent drug responses and compared the reactions of diseased versus healthy cells [2,6].

Our research builds on previous studies that emphasize the utility of hiPSC-CMs in both drug discovery and personalized medicine. hiPSC-CMs can be genetically matched to patients, providing unique insights into individual susceptibility to arrhythmogenic drugs and enabling the development of more precise therapeutic strategies [1,4]. This study further confirms the value of the MEA platform for identifying arrhythmic risks and reinforces the relevance of hiPSC-CMs as a preclinical model for cardiotoxicity testing.

METHODS

Cardiomyocyte differentiation

Three normal human induced pluripotent stem cell (iPSC) lines (CMC-006, CMC-011, CMC-016) and one LQTS hiPSC lines (DPHC01i-AR) were obtained from the Korea Stem Cell Bank and differentiated into cardiomyocytes (iPSC-CMs) using previously described methods, with detailed information on these cell lines provided in Table 1 [7-10]. Briefly, iPSCs were seeded onto a cell culture dish coated with hESC-qualified Matrigel (Corning). 10 μM Y-27632 (Tocris) was added 24 h after the initial seeding to enhance cell survival. The medium was changed daily, and iPSCs were cultured in StemMACS iPS-Brew XF (Miltenyi Biotec) until they reached approximately 90% confluency, which typically occurred after 4 days. On the first day of differentiation (Day 0), 8 μM CHIR99021 was added to the cardiomyocyte differentiation medium (CDM), consisting of RPMI1640 (Thermo Fisher Scientific), 500 μg/ml human albumin (Sigma-Aldrich), and L-ascorbic acid-2-phosphate (Sigma-Aldrich). The medium was replaced with CDM supplemented with 2 μM Wnt-C59 after 48 h, and the cells were cultured for another 48 h under these conditions. Medium was replaced with fresh CDM. On Day 5 and then refreshed every two days. The first signs of spontaneous contractions in the cells were observed between Day 6 and Day 10. To metabolically select and purify cardiomyocytes, the medium was replaced with glucose-free RPMI1640 medium (Thermo Fisher Scientific) containing 4 mM L-lactic acid for 4 days [11,12]. The purified iPSC-CMs were subsequently cultured in Advanced MEM medium (Thermo Fisher Scientific) supplemented with 100 ng/ml of 3,3',5-triiodo-L-thyronine (Sigma-Aldrich) and 1 μM dexamethasone (Stemcell Technologies), and the medium was changed every two days for 30 days [13]. Mature iPSC-CMs were dissociated into single-cell suspensions using TrypLE (Thermo Fisher Scientific) and seeded onto glass coverslips coated with 0.1% gelatin and MEA plates. Electrophysiological recordings for analysis were performed 48 h after seeding.

Gene expression *via* real time polymerase chain reaction (PCR)

Total RNA from hiPSC-CMs was isolated using TRIzol Reagent, according to the manufacturer's instructions. Human

Table 1. Information on four hiPSC lines

Cell line	Disease	Origin	Mutation	Information
CMC-006	-	Cord Blood Cell	-	[9]
CMC-011	-	Cord Blood Cell	-	[9]
CMC-016	-	Cord Blood Cell	-	Supplementary Figure 1
DPHCi-01	LQTS	Peripheral blood	KCNH2	[10]

hiPSC, human induced pluripotent stem cell; LQTS, long QT syndrome.

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Heart Total RNA (Takara Bio Inc. 636532) was purchased. The cDNA was reverse transcribed with a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). The cDNA (1 μl) was then mixed with 10 μl of FastStart Essential DNA Green Master Mixture (Thermo Fisher Scientific), 8 μl of distilled water, and forward and reverse primer (1 μl each). Primers were designed using Primer 3 for various genes, including glyceraldehyde 3phosphate dehydrogenase (*GAPDH*), troponin T2, cardiac type (*TNNT2*), myosin heavy chain 6 (*MYH6*), sodium voltage-gated channel alpha subunit 5 (*SCN5A*), potassium voltage-gated channel subfamily H member 2 (*KCNH2*), potassium voltage-gated channel subfamily D member 3 (*KCND3*), and calcium voltage-gated channel subunit alpha 1C (*CACNA1C*). Quantitative PCR (qPCR) were performed with the Light Cycler 96 system (Roche) under the following cycling conditions: 95°C for 10 min for initial denaturation, followed by 45 cycles of 95°C for 10 sec, 56°C for 10 sec, and 72°C for 10 sec. All qPCR were performed in triplicate to ensure accuracy and reproducibility. Gene expression levels were normalized to *GAPDH*, and the $2^{\Delta\Delta Ct}$ method was used for data analysis. Primer sequences are listed in Table 2.

Immunocytochemistry

Human iPSC-CMs were plated onto gelatin-coated cover slip and cultured for 5 days, fixed with 4% paraformaldehyde for 20 min at 4°C, permeabilized with 0.1% Triton X-100 for 10 min at room temperature, and blocked with Dulbecco's phosphate-buffered saline (DPBS) containing 5% normal goat serum for 30 min at room temperature. Subsequently, the cells were stained with antibodies against cardiac Troponin T (cTnT; 1:500, Abcam) and myosin heavy chain alpha (MHCα; 1:200, Abcam) diluted in 5% normal goat serum and incubated at 4°C overnight. The cells were washed three times for 10 min with DPBS and incubated with Goat anti-Rabbit IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 594 (Thermo Fisher Scientific), Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, and Alexa Fluor 488 (Thermo Fisher Scientific) antibodies for 1 h at room temperature. The cells were washed three times before counterstaining the nuclei with DAPI staining solution. Samples were visualized using a confocal microscope (LSM-700; Carl Zeiss) at the Cardiovascular and Metabolic Research Core Support Center at Inje University, South Korea.

Whole-cell patch clamp recording

Microglass patch pipettes (World Precision Instruments) were fabricated using a PC-100 puller (Narishige) to produce pipette resistances of approximately 2–2.5 MΩ when filled with internal solution. Whole-cell currents were amplified using an Axopatch 200B amplifier and digitized through a Digidata 1550B (Molecular Devices). The collected data was analyzed with pClamp 10.1 (Molecular Devices) and Origin 2016 (OriginLab).

Action potentials (AP) from hiPSC-CMs were recorded at 37°C ±0.5°C using an in-line heating system connected to a TC324C controller (Warner Instruments Inc.). The extracellular solution contained (in mM): 145 NaCl, 5.4 KCl, 1.8 CaCl2, 1 MgCl2, 5 glucose, and 10 HEPES, adjusted to pH 7.4 using NaOH. The internal solution consisted of (in mM): 130 K-aspartate, 20 KCl, 5 NaCl, 0.1 EGTA, 1 MgCl2, 3 MgATP, and 10 HEPES, with the pH adjusted to 7.2 using KOH.

MEA measurements and analysis

The hiPSC-CMs were seeded onto 96-well MEA plates precoated with fibronectin. After confirming spontaneous contractile activity, baseline parameters were obtained using the Axion MEA system: field potential duration (FPD), rate-corrected FPD (FPDc), beat period, and peak amplitude (Amp). Axion's Cardiac Analysis Tool was used to analyze the collected electrophysiological data. Drugs were applied at four concentrations, starting from 1x (the free clinical Cmax concentration) and increasing half-logarithmically to higher subsequent concentrations, as detailed in Table 3. The experimental protocol for this study was designed based on the drug concentrations reported by Millard *et al*. [14]. Using this approach, the electrophysiological responses of hiPSC-CMs to E4031, nifedipine, mexiletine, JNJ303, ranolazine, quinidine, flecainide, and moxifloxacin were evaluated. FPD was recorded before and after a 30-min drug exposure period to ensure proper equilibration. Concentration-dependent changes in electrophysiological parameters were analyzed to assess the

Table 2. Real-time PCR primer sequences

Gene	Forward sequence (5′→3′)	Reverse sequence (5′→3′)	Size (bp)	Gene Bank No.
GAPDH	gtatgacaacagcctcaaga	gtagaggcagggatgatgt	216	NM_002046
TNNT2	gggttacatccagaagacag	gttatagatgctctgccaca	163	NM_000364
MYH6	agtatgaggagtcgcagtct	cacattctttcctccttctc	194	NM_002471
SCN5A	tcaccacctacatcatcatc	gacaggaccgaatactcaat	190	AY038064
KCNH2	cctccatcaaggacaagtat	gaagatgctagcgtacatga	155	AF363636
KCND3	ctactacatcggtctggtca	tgagggagaagagaagaaag	179	NM_004980
KCNQ1	tactttgtgtacctggctga	agatggcaaagacagagaag	181	AY114213
CACNA1C	ccatctacaactaccgtgtg	accacgtaccacactttgta	247	NM_1994603

Table 3. Test concentrations and preparation of drugs for MEA analysis of drug responses

Drug	Test concentration	μM	Stock	10X-media (Stock/media μl)
E4031	#1	0.003	#3	20 / 180
	#2	0.01	#4	20 / 180
	#3	0.03	0.01 mM	1 / 299
	#4	0.1	0.01 mM	4 / 396
Nifedipine	#1	0.01	#3	20 / 180
	#2	0.03	#4	20 / 180
	#3	0.1	0.3 mM	1 / 299
	#4	0.3	0.3 mM	4 / 396
Mexiletine	#1	1	#3	20 / 180
	#2	3	#4	20 / 180
	#3	10	30 mM	1 / 299
	#4	30	30 mM	4 / 396
JNJ303	#1	0.01	#3	20 / 180
	#2	0.03	#4	20 / 180
	#3	0.1	0.3 mM	1 / 299
	#4	0.3	0.3 mM	4 / 396
Flecainide	#1	3	#3	20 / 180
	#2	10	#4	20 / 180
	#3	30	3 mM	1 / 299
	#4	100	3 mM	4 / 396
Moxifloxacin	#1	3	#3	20 / 180
	#2	10	#4	20 / 180
	#3	30	100 mM	1 / 299
	#4	100	100 mM	4 / 396
Quinidine	#1	0.3	#3	20 / 180
	#2	1	#4	20 / 180
	#3	3	10 mM	1 / 299
	#4	10	10 mM	4 / 396
Ranolazine	#1	1	#3	20 / 180
	#2	3	#4	20 / 180
	#3	10	30 mM	1 / 299
	#4	30	30 mM	4 / 396

MEA, multi-electrode array.

impact of each drug on the cardiomyocytes' electrical activity.

Data analysis was conducted using Axion's Cardiac Analysis Software with percent change in FPD and FPDc calculated as follows: % change in FPD = (post-drug FPD / baseline FPD) * 100, with standard deviation (± SD) provided for each value. This calculation allows direct comparisons of electrophysiological effects between treated samples and untreated controls.

In line with the CiPA core protocol that ensures reproducibility across multiple testing sites, standardized cell preparation methods, experimental procedures, and analysis metrics were implemented. While the protocol provides flexibility to accommodate different platforms and cell types, it maintains consistency in key experimental elements to ensure comparability across studies [14].

RESULTS

Molecular and functional characterization of differentiated cardiomyocytes

Four different hiPSC lines (CMC-006, CMC-011, CMC-016, and DPHC01i-AR) successfully *in vitro* differentiated to cardiomyocytes (Supplementary Video 1–4). Then, the hiPSC-CMs were characterized to confirm their cardiomyocyte-specific properties. To assess differentiation efficiency, qPCR analysis was conducted to measure the expression levels of cardiomyocyte-specific markers. The expression of *TNNT2* and *MYH6*, two well-established markers of cardiac differentiation, was significantly elevated in all four cell lines. The data showed that these markers were upregulated by approximately 300-folds compared to undifferentiated pluripotent stem cells, indicating robust differentiation into cardiomyocytes (Fig. 1).

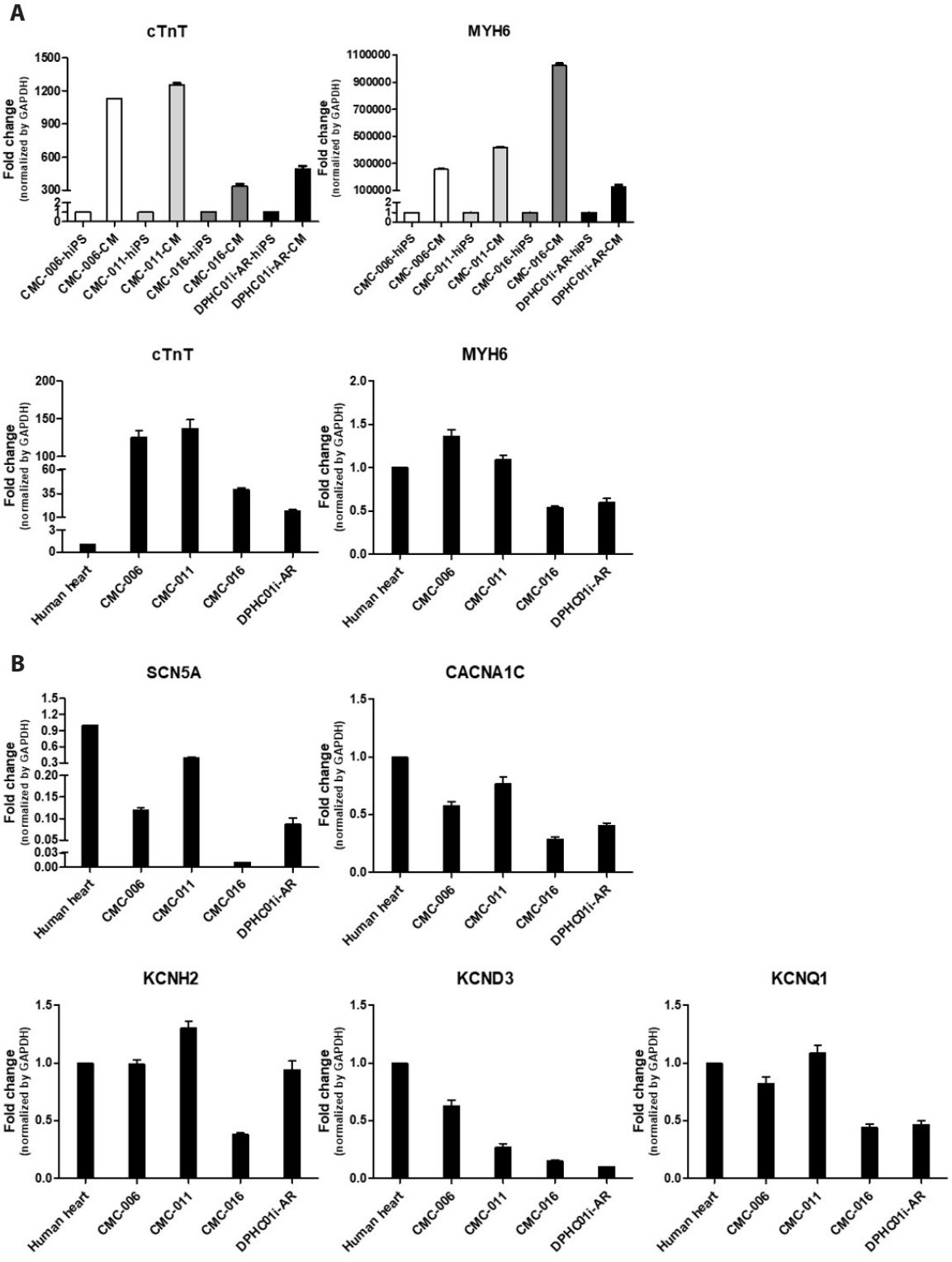


Fig. 1. Gene expression analysis of cardiomyocyte-specific and ion channel genes in control and hiPSC-CM lines by RT-PCR. (A) Cardiomyocyte-specific markers: The expression levels of *TNNT2* and *MYH6* were measured in control cells and four hiPSC-CM lines (CMC-006, CMC-011, CMC-016 derived from healthy individuals, and DPHC01i-AR derived from a patient with LQTS). The results are shown as fold changes normalized to the housekeeping gene *GAPDH*. The CMs of the four cell lines were normalized to their respective hiPS cells, with the hiPS cells set to 1 (upper panel). All hiPSC-CM cell lines exhibited significant upregulation of *TNNT2* and *MYH6* compared to the control, indicating successful differentiation into cardiomyocytes. (B) Ion channel gene expression: Key ion channel gene (*SCN5A*, *KCNH2*, *KCND3*, *KCNQ1*, and *CACNA1C*) expression levels were analyzed in the same cell lines. *SCN5A*, *KCNH2*, and *KCNQ1* showed comparable expression levels across all cell lines, whereas *KCND3* and *CACNA1C* exhibited variability in expression. Values are presented as mean ± SD. hiPSC-CM, human induced pluripotent stem cell-derived cardiomyocyte; cTnT, cardiac Troponin T; LQTS, long QT syndrome.

When compared to adult human heart tissue, the differentiated cardiomyocytes displayed a 10-fold increase in *TNNT2* and *MYH6* expression, suggesting a high degree of purity and cardiomyocyte identity in the differentiated cells. Immunofluorescence staining further confirmed the presence of cardiomyocyte-specific proteins, including cTnT and SA-Actinin, in all four cell lines. Staining results showed clear sarcomeric structures, typical of mature cardiomyocytes, and co-localization of cTnT and MHC, supporting the successful differentiation of these cells. Moreover, spontaneous contractile activity was observed in all lines, further demonstrating the functional characteristics of the differentiated cardiomyocytes (Fig. 2).

Electrophysiological profiling of hiPSC-derived cardiomyocytes using patch clamp analysis

Electrophysiological properties were recorded from the four hiPSC-CM cell lines using whole cell patch clamps and analyzed. These parameters included action potential (AP), beat period, APD50, APD80, maximum upstroke velocity, Amp, and maximum diastolic potential to fully assess the electrical function of the cells (Fig. 3A).

APs were categorized into three types: ventricular-like, atrial-like, or nodal-like, based on their characteristic shape and parameters. Notably, more than 70% of CMC-006, CMC-011, and CMC-016 cells exhibited ventricular-like APs, suggesting that these cells closely mimic the electrical properties of ventricular cardiomyocytes (Fig. 3B, C). The remaining 30% of cells in these lines showed atrial-like or nodal-like APs (Table 4). In contrast, the DPHC01i-AR cell line, derived from a patient with LQTS, exhibited prolonged APs with distinct arrhythmic characteristics, consistent with the presentation of LQT in the source tissue. A detailed analysis of AP parameters revealed diffrences between the control and DPHC01i-AR cell lines. DPHC01i-AR exhibited prolonged APD80 and APD50 values, reflecting an increased susceptibility to arrhythmias. Interestingly, in a previous study using the same c.453delC mutation in the *KCNH2* gene, patient-derived hiPSC-CMs exhibited a similarly prolonged AP duration and dis-

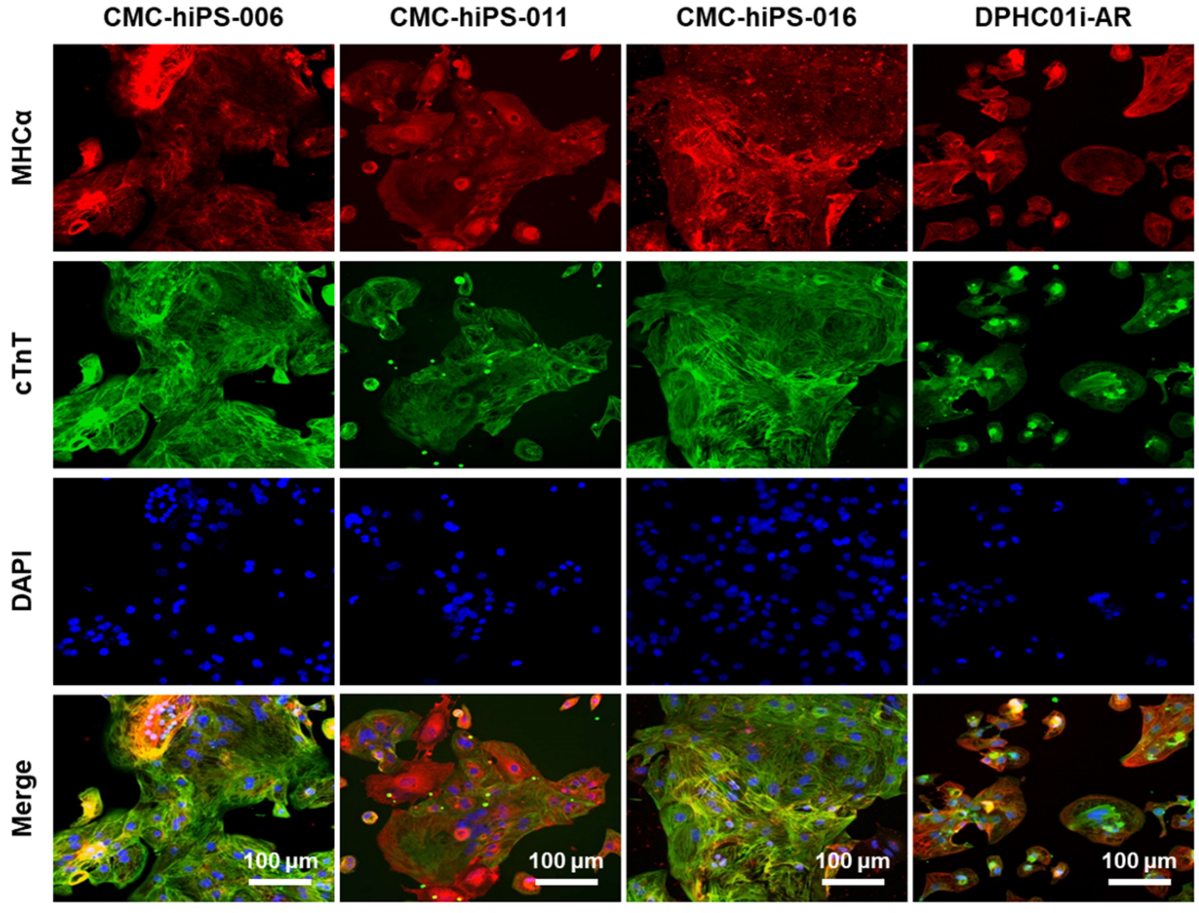


Fig. 2. Immunocytochemical analysis of cardiomyocyte markers in hiPSC-CMs. Immunofluorescence staining was performed to detect cardiomyocyte-specific proteins in the four hiPSC-CM lines (CMC-006, CMC-011, CMC-016, and DPHC01i-AR). Myosin heavy chain α (MHCα) is shown in red, cardiac Troponin T (cTnT) is shown in green, and nuclei are stained with 4′,6-diamidino-2-phenylindole (DAPI) in blue. The merged images display co-localization of these markers, confirming the expression of cardiomyocyte-specific proteins in all cell lines. The structural organization and marker expression exhibit slight variations across the cell lines, indicating successful differentiation with some heterogeneity in morphology. Scale bar: 100 μm. hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes.

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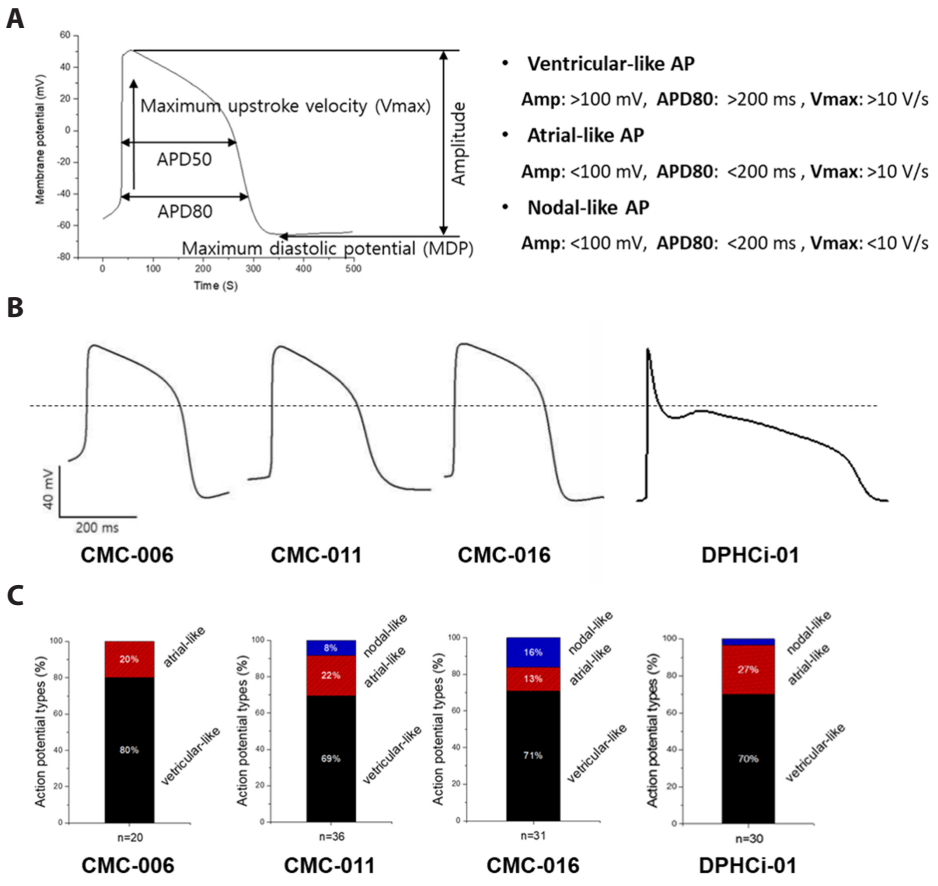


Fig. 3. Analysis of action potentials (AP) in hiPSC-CMs. (A) AP characteristics: A schematic showing the AP parameters measured in hiPSC-CMs, including maximum upstroke velocity (Vmax), amplitude (Amp), maximum diastolic potential (MDP), APD50, and APD80. (B) Representative AP: AP traces recorded from the CMC-006, CMC-011, CMC-016, and DPHC01i-AR cell lines using patch-clamps. Each trace displays the characteristic features of either ventricular-like or nodal-like APs. The DPHC01i-AR cell line exhibits prolonged APD with abnormal repolarization, reflective of its LQT background. (C) Proportions of AP types: The percentage distribution of ventricular-like, atrial-like, and nodal-like APs in each cell line (CMC-006, CMC-011, CMC-016, and DPHC01i-AR). A majority of cells in each cell line exhibit ventricular-like APs. hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes; LQT, long QT.

Table 4. Analysis of action potential parameter

Cell line	Beat period (ms)	APD80 (ms)	APD50 (ms)	Vmax (V/s)	Amp (mV)	MDP (mV)
CMC-006 (n = 16)	1,441.9 ± 809.4	249.5 ± 37.8	219.0 ± 35.8	20.1 ± 12.1	115.1 ± 3.0	–73.8 ± 2.0
CMC-011 (n = 25)	2,017.2 ± 768.3	288.8 ± 66.4	261.2 ± 64.7	45.7 ± 5.5	114.7 ± 6.1	–76.1 ± 2.5
CMC-016 (n = 22)	1,944.3 ± 874.9	211.5 ± 16.4	192.8 ± 19.2	22.6 ± 7.2	109.6 ± 3.5	–69.1 ± 2.6
DPHCi-01 (n = 30)	2,700.1 ± 2,348.9	276.3 ± 62.0	221.0 ± 73.8	31.7 ± 15.4	105.5 ± 11.6	–69.8 ± 5.9

Values are presented as mean ± SEM. Vmax, maximum upstroke velocity; Amp, amplitude; MDP, maximum diastolic potential.

tinct arrhythmic features [8]. These findings provide valuable insights into the electrophysiological behavior of the DPHC01i-AR cell line compared to control cell lines and highlight the utility of these hiPSC-CMs for modeling cardiac disease states.

Assessment of drug-induced electrophysiological responses in hiPSC-CMs using MEA

The responses of the four hiPSC-CM cell lines to arrhythmogenic drug exposure were evaluated using an MEA system. The eight arrhythmogenic drugs tested in this study (E4031, nifedipine, mexiletine, JNJ303, flecainide, moxifloxacin, quinidine, and ranolazine) were selected based on their known ability to affect cardiac electrophysiology and induce arrhythmias. Electrophysiological signals such as FPD, FPDc, beat period, and the peak Amp of depolarization were monitored in response to various drug concentrations and analyzed using the Cardiac Analysis Tool.

In the control cell lines (CMC-006, CMC-011, CMC-016), E4031 and nifedipine produced dose-dependent changes in FPD, with results consistent with those observed in global cell line studies. Mexiletine, a sodium channel blocker, exhibited a predictable shortening of FPD, aligning with data from four other global sites, reinforcing the reproducibility and reliability of the MEA platform in these cell lines (Fig. 4–7). In contrast, JNJ303, a calcium channel modulator, did not induce significant FPD changes in any cell line, consistent with previously reported global data that show minimal arrhythmic effects in response to this compound.

The DPHC01i-AR cell line exhibited heightened sensitivity to several of the drugs tested. Exposure to E4031, a known hERG channel blocker, induced a longer FPD than control cell lines, re-

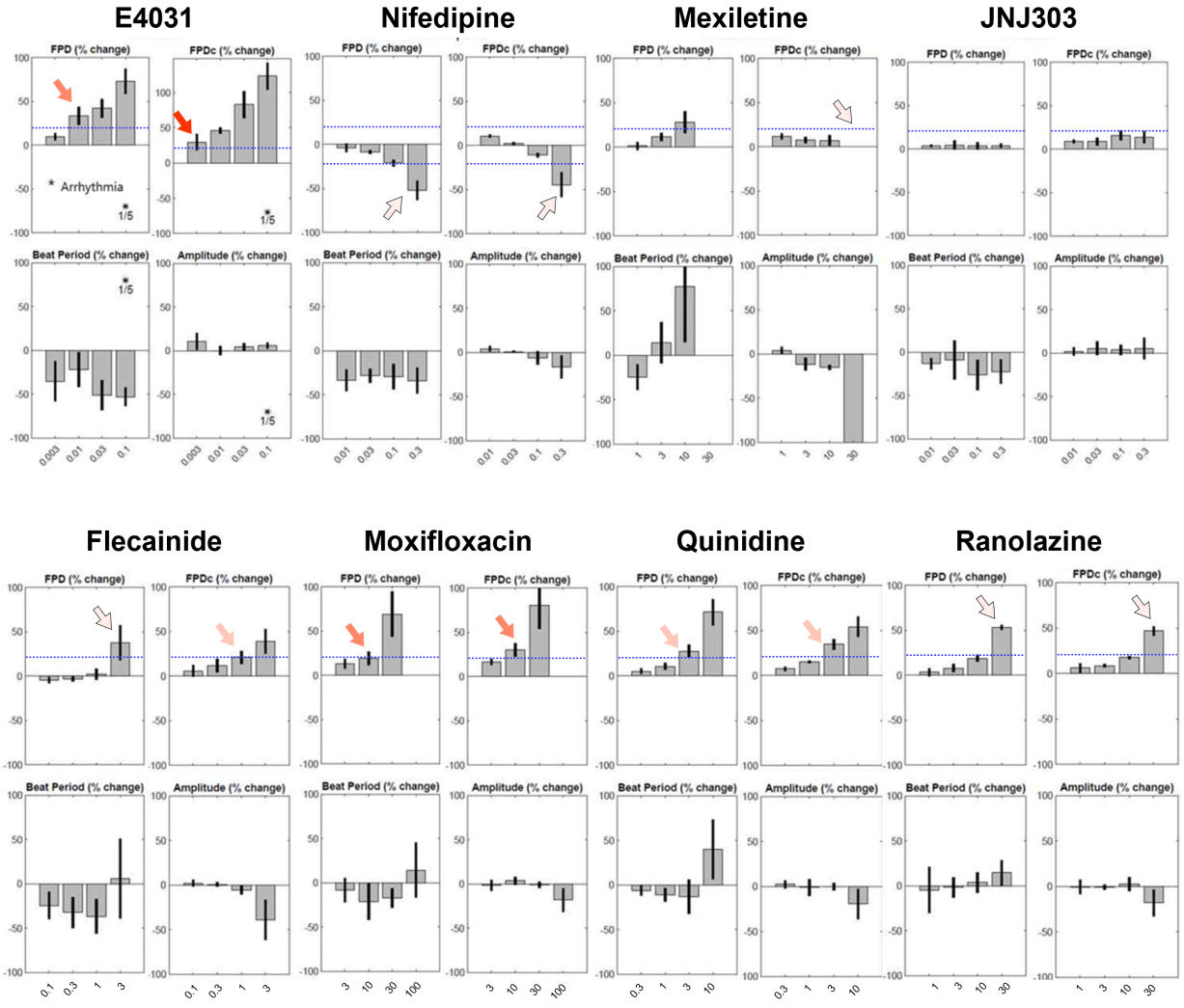


Fig. 4. Multi-electrode array (MEA) analysis of electrophysiological responses to eight arrhythmogenic drugs in hiPSC-CMs (CMC-006). MEA analysis showing changes in field potential duration (FPD), corrected FPD (FPDc), beat period, and amplitude (expressed as percentages) in response to varying concentrations of eight drugs: E4031, nifedipine, mexiletine, JNJ303, flecainide, moxifloxacin, quinidine, and ranolazine. The arrhythmic beats observed with E4031 are represented as the number of occurrences relative to the total number of repetitions (observations/repetitions). The blue dot line indicates the 20% change threshold for FPD and FPDc, which is considered biologically relevant for cardiotoxicity. The arrow indicates the FPD20 or FPDc20, with the depth of the red color representing the decrease in FPD20 or FPDc20. hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes.

flecting the drug's propensity to exacerbate arrhythmias in DPHC01i-AR cells. Flecainide, moxifloxacin, quinidine, and ranolazine also elicited dose-dependent responses from DPHC01i-AR cells, and these effects were consistent with global benchmarks.

To compare the drug-induced responses of hiPSC-CMs across different cell lines, the parameters FPD20 and FPDc20 were defined as the drug treatment concentrations that induce more than a 20% change in FPD and FPDc, respectively (blue dot line in Fig. 4-7). These values are denoted by red-colored arrows, with arrows of greater depth representing lower FPD20 or FPDc20 (red arrows in Fig. 4-7). Additionally, the values were subsequently compared for each drug and cell line (Fig. 8). The normal cell lines exhibited FPD20 and FPDc20 changes comparable to those observed in global cell line studies [14]. Notably, the DPHC01i-AR arrythmia cell line exhibited a lower FPD20 for E4031 and mexiletine in the FPD20 analysis, and for nifedipine in the FPDc20 analysis, compared to the normal cell lines. Furthermore, mexiletine caused less than a 20% change in FPD across various treatment concentrations in the normal cell lines. However, in the DPHC01i-AR cell line, it induced more than a 20% change even at the lowest treatment concentration (red arrow at Fig. 7). The arrow indicates the FPD20 or FPDc20, with the depth of the red color representing the decrease in FPD20 or FPDc20. These findings suggest that hiPSC-CMs derived from patients with arrhythmia are particularly sensitive to drug-induced arrhythmias, making them a valuable model for preclinical cardiotoxicity testing. Compara-

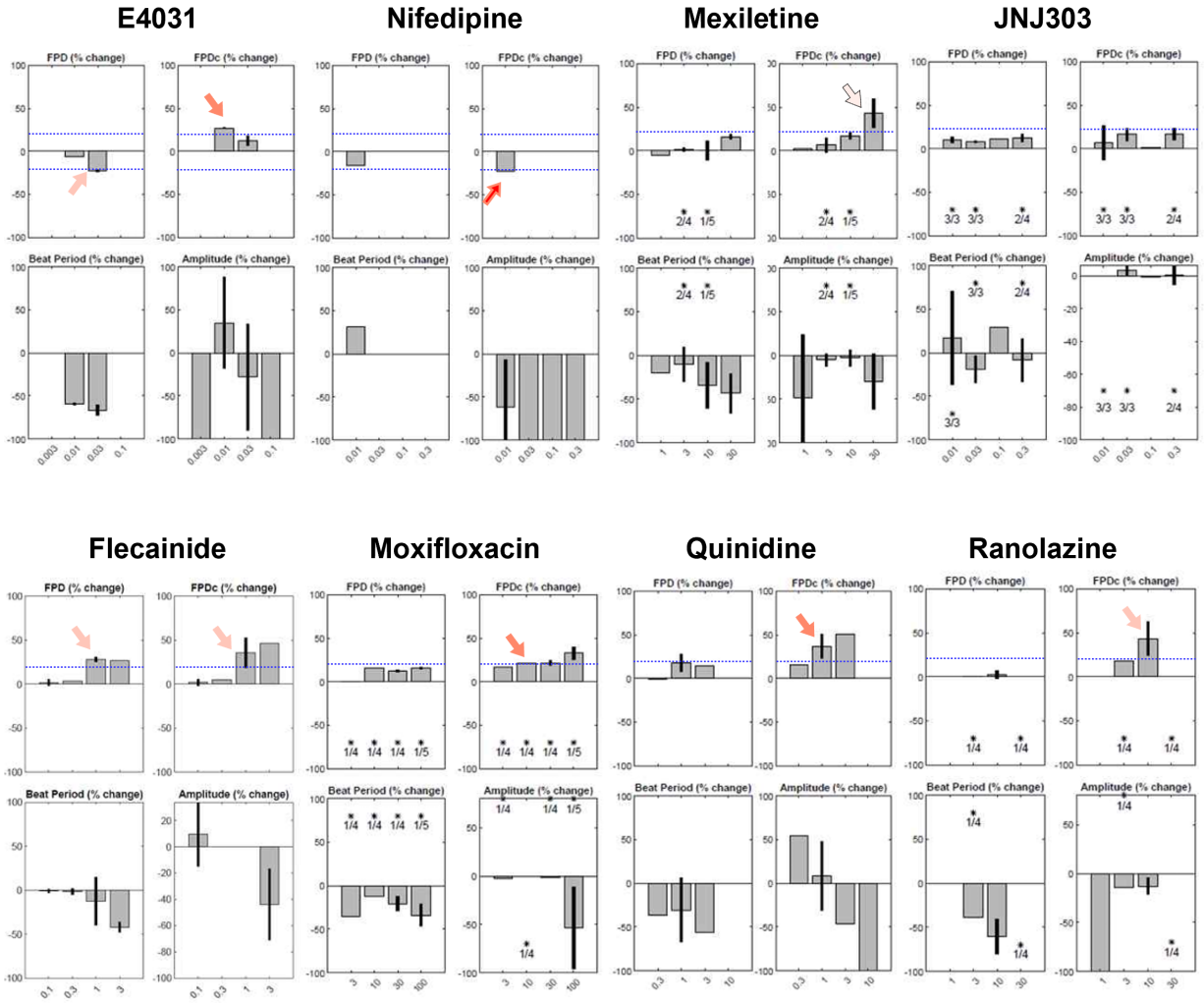


Fig. 5. Multi-electrode array (MEA) analysis of electrophysiological responses to eight arrhythmogenic drugs in hiPSC-CMs (CMC-011). MEA evaluation depicting the percentage alterations in field potential duration (FPD), corrected FPD (FPDc), beat period, and amplitude in response to varying concentrations of eight arrhythmogenic drugs: E4031, nifedipine, mexiletine, JNJ303, flecainide, moxifloxacin, quinidine, and ranolazine. The arrhythmic beats observed with mexiletine, JNJ303, moxifloxacin, and ranolazine are represented as the number of occurrences relative to the total number of repetitions (observations/repetitions). The blue dot line indicates the 20% change threshold for FPD and FPDc. The arrow indicates the FPD20 or FPDc20, with the depth of the red color representing the decrease in FPD20 or FPDc20. hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes.

tive analyses of drug responses between control cell lines and the patient-derived cell line highlight the utility of hiPSC-CMs in detecting arrhythmogenic risks in normal and diseased cardiac cells. These results further validate the relevance of using hiPSC-CMs and MEA platforms for preclinical cardiotoxicity assessments.

DISCUSSION

The development of hiPSC-CMs has significantly advanced the field of preclinical cardiotoxicity testing. In this study, we evaluated the electrophysiological responses of hiPSC-CMs to a panel of eight drugs with varying arrhythmogenic characteristics, demonstrating the value of this model for predicting drug-induced cardiac risks [15]. This study reinforces the belief that hiPSC-CMs are a relevant tool that bridges a gap between preclinical models and human cardiac physiology, offering a reliable method for detecting drug-induced cardiotoxicity earlier in the drug development process [1].

The ability of hiPSC-CMs to naturally express a comprehensive array of cardiac ion channels makes them a more physiologically relevant platform compared to heterologous expression systems that rely on overexpression of individual channel proteins. The dose-dependent effects of drugs like E4031 and nifedipine, observed in control cell lines, highlight the reliability of hiPSC-CMs for replicating known drug responses seen in human cardiac tissue. These findings align with previous studies that support the

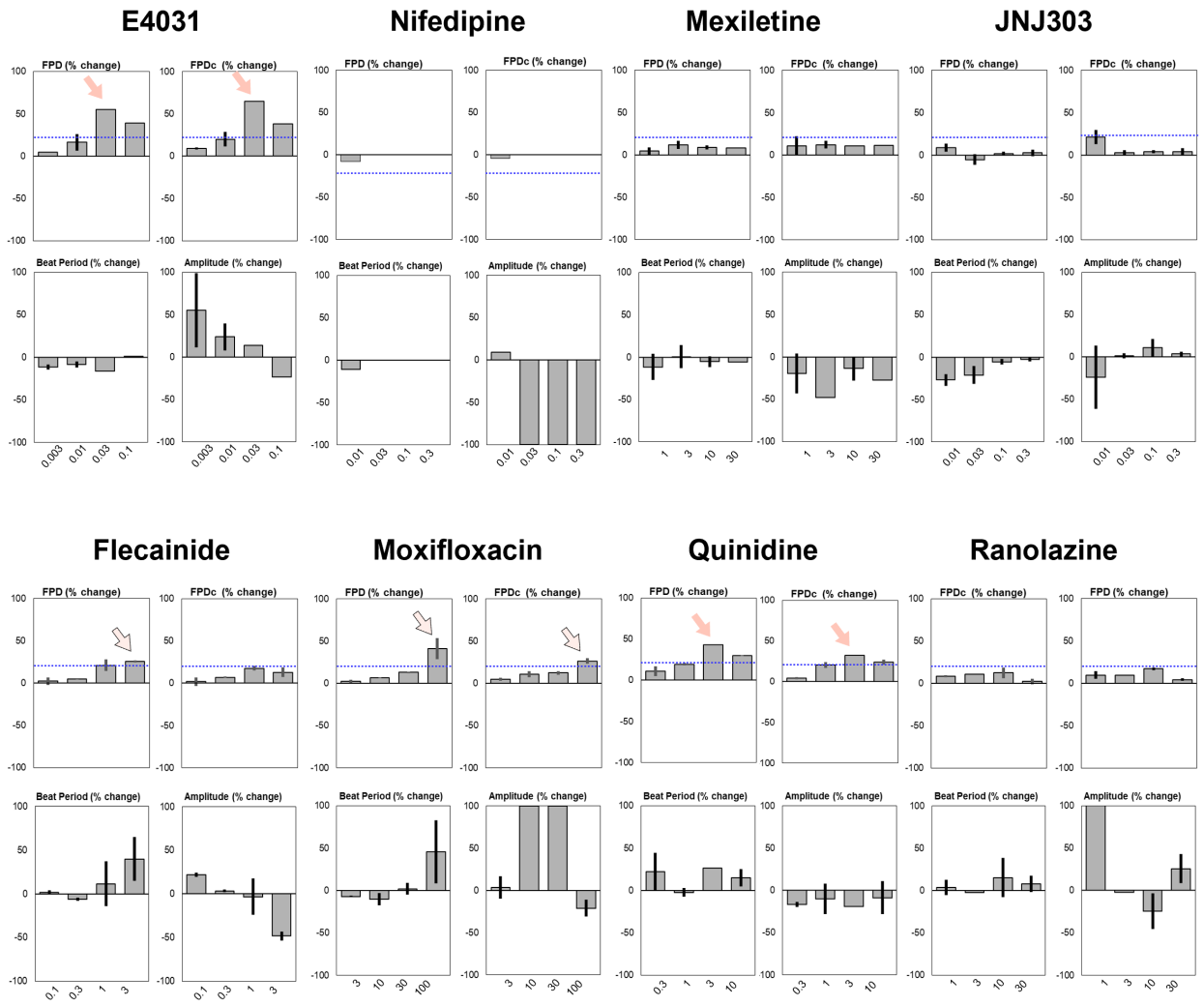


Fig. 6. Multi-electrode array (MEA) study of drug-induced electrophysiological effects in hiPSC-CMs (CMC-016). MEA study illustrating the percentage changes in electrophysiological parameters, including field potential duration (FPD), corrected FPD (FPDc), beat period, and amplitude, observed after exposure to eight drugs: E4031, nifedipine, mexiletine, JNJ303, flecainide, moxifloxacin, quinidine, and ranolazine. The blue dot line indicates the 20% change threshold for FPD and FPDc. The arrow indicates the FPD20 or FPDc20, with the depth of the red color representing the decrease in FPD20 or FPDc20. hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes.

use of hiPSC-CMs for assessing drug safety and their integration into regulatory initiatives like CiPA [4,14,16].

Moreover, the heightened sensitivity of the DPHC01i-AR cell line to E4031 and other drugs demonstrates the utility of disease-specific hiPSC-CMs for modeling genetic disorders associated with arrhythmias. The distinct electrophysiological responses observed in the DPHC01i-AR cell line not only reflect the underlying hERG channel mutation but also offer valuable insights into how patient-specific models can be used to predict individualized drug responses [8]. This supports the growing recognition of hiPSC-CMs as an essential tool for precision medicine, enabling more tailored drug safety evaluations for patients with genetic predispositions [14].

Despite the promising capabilities of hiPSC-CMs, there are still challenges that need to be addressed. One major limitation is the relatively immature state of hiPSC-CMs compared to adult cardiomyocytes. This difference may affect their ability to fully replicate the electrophysiological properties of mature heart cells [13]. Immaturity can affect AP durations and ion channel expression levels, potentially impacting the translation of in vitro findings to clinical outcomes. Mechanical and electrical stimulation have been shown to enhance contractility and electrophysiological properties, while metabolic conditioning promotes mitochondrial maturation and energy metabolism [17]. 3D culture systems can mimic the *in vivo* cardiac microenvironment, thereby promoting the structural and functional maturation of hiPSC-CMs. Incorporating this approach into cardiotoxicity assays could enhance hiPSC-CM-based models, enabling them to more accurately represent adult human cardiomyocytes. Additionally, the variability between different hiPSC-CM lines presents a challenge for standardizing drug testing protocols. The differences in drug responses we observed between control cell lines are likely attrib-

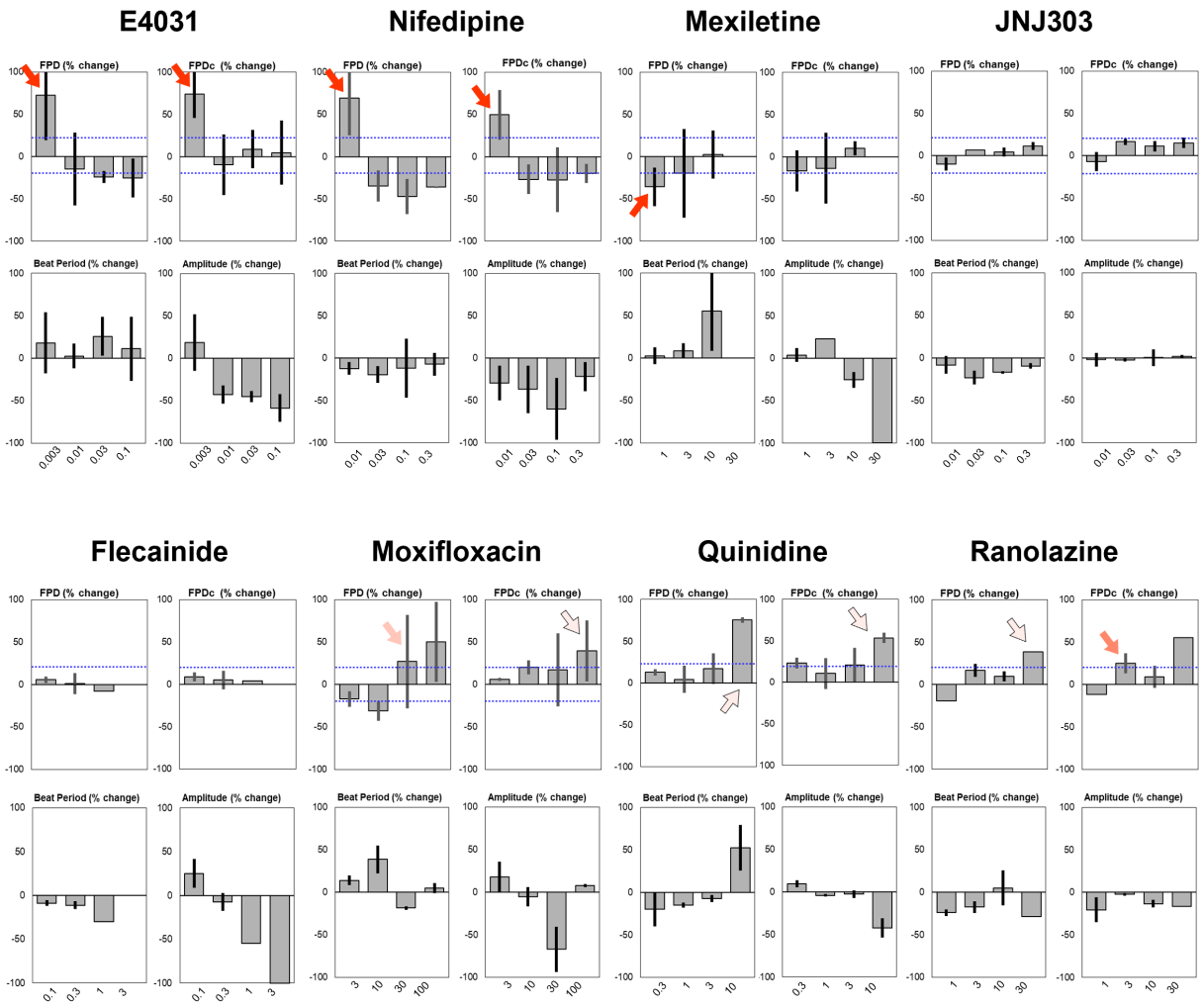


Fig. 7. Multi-electrode array (MEA) analysis of electrophysiological changes in response to drugs in hiPSC-CMs (DPHC01i-AR). MEA analysis highlighting the percentage changes in field potential duration (FPD), corrected FPD (FPDc), beat period, and amplitude, triggered by varying concentrations of eight drugs: E4031, nifedipine, mexiletine, JNJ303, flecainide, moxifloxacin, quinidine, and ranolazine. The blue dot line indicates the 20% change threshold for FPD and FPDc. The arrow indicates the FPD20 or FPDc20, with the depth of the red color representing the decrease in FPD20 or FPDc20. hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes.

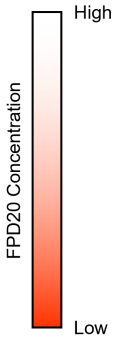
utable to variations in differentiation efficiency, cell maturation, ion channel expression, and metabolic activity [13]. A concerted effort toward the development of standardized differentiation protocols and more consistent hiPSC-CM lines will be necessary to improve reproducibility across studies and laboratories [14].

Furthermore, some normal hiPSC-CM lines demonstrate a tendency to exhibit cardiotoxic responses to anti-arrhythmic drugs, despite being derived from healthy individuals. While normal cell lines typically demonstrate low or negligible cardiotoxicity, consistent with expectations from preclinical models, certain cell lines exhibit responses similar to those of arrhythmia patient-derived cell lines when exposed to drugs such as E4031 and nifedipine. These findings highlight the dependency on specific cell lines in cardiotoxicity evaluations using hiPSC-CMs. To enhance the accuracy of predictions for drug-induced cardiotoxicity, it is crucial to establish robust evaluation criteria based on comprehensive toxicity assessment data from both normal and arrhythmia patient-derived hiPSC-CM lines.

Integrating advanced computational tools, such as deep learning algorithms, into hiPSC-CM assays holds great potential for enhancing cardiotoxicity predictions. Recent studies show that deep learning models can identify complex electrophysiological patterns linked to proarrhythmic events with greater precision than traditional methods. This approach improves the detection of subtle signals and allows for large-scale data analysis, making hiPSC-CM assays more scalable for high-throughput drug screening [18]. Such integration not only accelerates and streamlines the drug discovery process but also enables a more effective and cost-efficient assessment of the proarrhythmic potential of various drugs. Additionally, combining experimental data with computer model-based simulations provides a more robust validation framework for predicting the electrophysiological proper-

A

Drug \ Cell line	CMC-006	CMC-011	CMC-016	DPHC01i-AR
E4031	0.01	0.03	0.03	0.003
Nifedipine	0.3	-	-	0.01
Mexiletine	-	-	-	1
JNJ303	-	-	-	-
Flecainide	3	1	3	-
Moxifloxacin	10	-	100	30
Quinidine	3	-	3	10
Ranolazine	30	-	-	30



B

Drug \ Cell line	CMC-006	CMC-011	CMC-016	DPHC01i-AR
E4031	0.003	0.01	0.03	0.003
Nifedipine	0.3	0.01	-	0.01
Mexiletine	30	30	-	-
JNJ303	-	-	-	-
Flecainide	1	1	-	-
Moxifloxacin	10	10	100	100
Quinidine	3	1	3	10
Ranolazine	30	10	-	3

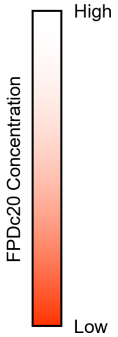


Fig. 8. Comparison of drug-induced responses in hiPSC-CMs across cell lines. (A) Heatmap showing the minimum concentrations that induce a change in FPD of more than 20% (FPD20). (B) Heatmap showing the minimum concentrations that induce a change in FPDc of more than 20% (FPDc20). The depth of red color represents FPD20 or FPDc20 concentrations, from the lowest to the highest, respectively. White indicates that no concentration tested induced a change in FPD or FPDc of over 20%. hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes; FPD, field potential duration; FPDc, corrected FPD.

ties of drugs [19]. For instance, machine learning algorithms can preemptively predict the risk profiles of specific drugs, enabling a hybrid approach that combines experimental methodologies with computational models. Leveraging these technologies could refine the predictive power of hiPSC-CMs, broadening their role in drug discovery and personalized medicine [3]. We plan to further investigate the application of these computational tools in combination with hiPSC-CM models to improve the accuracy and personalization of cardiotoxicity screening. This ongoing research will focus on optimizing algorithms for the specific characteristics of patient-derived cardiomyocytes, with the ultimate goal of enhancing preclinical drug testing and patient safety.

In conclusion, this study demonstrates the utility of hiPSC-CMs as a model for preclinical cardiotoxicity testing. The results highlight the ability of hiPSC-CMs to detect drug-induced electrophysiological changes and the promise of hiPSC-CMs to improve drug safety evaluations, especially when combined with advanced analytical techniques. As hiPSC-CMs continue to mature and their limitations are addressed, they are likely to play a central role in future drug safety testing frameworks, offering a more accurate and patient-specific assessment of cardiotoxic risks.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary data including four videos can be found with this article online at https://doi.org/10.4196/kjpp.24.413

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