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The Agilent xCELLigence RTCA CardioECR System

A multiparametric functional analysis instrument for cardiac safety and toxicity assessment

Authors

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Abstract

The Agilent xCELLigence RTCA CardioECR system combines simultaneous
measurement of field potential signal using extracellular recording (ECR) electrodes
and contractile properties using impedance sensors for functional assessment
and viability of beating cardiomyocytes. The real-time multiplexed evaluation of
human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) using
the CardioECR system allows for a suitable assay platform to predict drug-induced
proarrhythmia, contractile liability, and chronic toxicity of drugs under development.

Introduction

The heart is one of the most common organs for adverse effects of drugs.[1] Drug-induced cardiac toxicities, including structural impairment, adverse contractile modulation, and life-threatening polymorphic ventricular tachyarrhythmia, known as torsades de pointes (TdP), are major concerns of cardiac safety assessment.

The potential of drug-induced cardiac arrhythmia has increasingly resulted in warning and withdrawal of numerous drugs from the market. Additionally, many compounds in pharmaceutical industry pipelines were terminated in the late preclinical or clinical development stages due to cardiac risks, particularly proarrhythmic liability.[2] A core battery of preclinical assays has been developed and currently used to predict proarrhythmic risk of new chemical entities, including the hERG assay performed in recombinant cell lines that exogenously express the ion channel of interest; the *in vitro* repolarization assay for measurement of action potential duration (APD); and *in vivo* assays for assessing changes in the QT interval of ECG obtained from non-rodent animals. Most recently, the *in vitro* data generated by the Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative demonstrates that the utility of human iPSC-CMs in conjunction with multi-electrode array (MEA) technology within the CiPA paradigm could predict TdP risk categories with reasonable accuracy (AUC >0.8) and manageable variability induced by experimental approaches and techniques.[3]

Proarrhythmic risk is only one aspect of cardiotoxicity. Drug-induced cardiac toxicity also includes structural impairment, such as changes in cardiac morphology, myocardial degeneration, or necrosis.[1] In contrast to proarrhythmic liability, which can be evaluated using short-term assays (e.g., patch clamp), structural impairment may require long-term assays due to the high possibility of adverse events only occurring after chronic treatment, such as those caused by anthracycline chemotherapeutic drugs.

In addition to drug-induced structural damage, changes in cardiac contractility emerging either as a primary effect of the drug on cardiac function or as a consequence of cardiac lesions should also be thoroughly addressed. The dog cardiomyocyte optical assay, which quantifies contraction velocity and sarcomere shortening in electrically paced adult dog cardiomyocytes using an optical microscopy system, has been validated and adopted as an *in vitro* assay for assessment of cardiac contractility.[4] Although the assay has adequate predictivity, its insufficient throughput hampers the process of implementing it in early drug screening.[5]

Taken together, there is an urgent need to develop new *in vitro* models and assay systems to assess human cardiac risk during the front-line screening phase. The ideal model would consist of a robust, medium- to high-throughput cellular system resembling human cardiomyocytes to the highest possible extent, which is also scalable and amenable to screening multiple targets in the heart.

In this application note, we used the xCELLigence RTCA CardioECR system (CardioECR system) in conjunction with hiPS-CMs for the assessment of preclinical cardiac risks. The CardioECR system is the first platform to combine impedance and field potential (FP) recording for simultaneous and noninvasive measurement of integrated ion channel activity and contractility of cardiomyocytes in a microplate format. The system allows comprehensive evaluation of hiPSC-CMs in terms of both acute effects on cardiac function, such as electrophysiology and contractility, and chronic effects on cardiac structure and viability.

Assay principle

The xCELLigence RTCA CardioECR system is a dual-mode instrument that includes both simultaneous monitoring of hiPSC-CM viability, contraction, and FP in real time as well as directed electrical pacing of hiPSC-CMs. It consists of four components: a CardioECR control unit (laptop); CardioECR analyzer; CardioECR station; and E-plate CardioECR 48 (CardioECR plate) (Figure 1A). Two sets of electrodes, interdigitated impedance (IMP) microelectrode arrays as well as two individual field potential electrodes, are integrated into the bottom of each well of the CardioECR plate (Figure 1B). Similar to other xCELLigence platforms, the CardioECR system uses IMP electrodes to measure cellular impedance, which is affected by the number of cells covering the electrode, the morphology of the cells, and the degree of the cell attachment. The fast sampling rate of IMP measurement (2 ms) allows capturing temporal

E-Plate CardioECR 48

A

B

Two separate sets of electrodes

IMP

ECR/FP
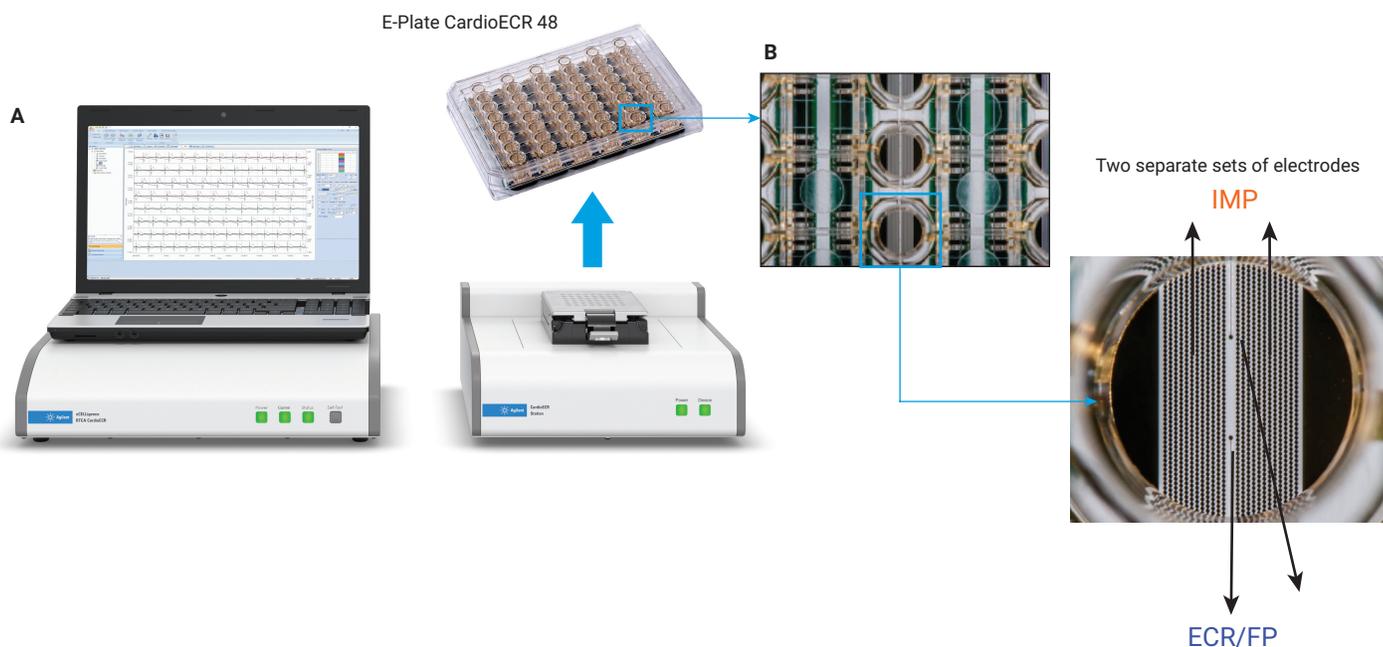
Figure 1. (A) The Agilent xCELLigence RTCA CardioECR system consists of four components: control unit/laptop, CardioECR analyzer (left), CardioECR station (right), and E-plate CardioECR 48. (B) A close-up image of E-plate CardioECR 48. Inset: a close-up of the wells reveals the layout of the electrodes, impedance (IMP) electrode assays, and two field potential (FP) electrodes.

rhythmic changes in cell morphology and degree of cell attachment to the plate associated with contraction of cardiomyocytes. Therefore, the physical contraction of cardiomyocytes is monitored and recorded in real time with very high temporal resolution. Additionally, the millisecond time resolution can be performed at regular intervals over a prolonged duration of time to provide beating information as well as information regarding the overall viability of the cardiomyocytes in real time. Furthermore, two FP electrodes are used to measure integrated ion

channel activities at a data acquisition rate of 10 kHz simultaneously with IMP recording via IMP electrodes.

One of the other critical features of the CardioECR system is its ability to electrically pace cardiomyocytes. During electrical pacing, electrical pulses are directly applied to the cells through the IMP electrodes. For most cardiomyocytes, the length of each electrical pulse employed by IMP electrode is less than 2 ms, which allows the contractile activities of cells to be immediately captured and recorded while the cells are being paced by IMP

electrodes. The optimal conditions for electrical pacing are dependent on the cell type, the inherent beating frequency, and the experimental context. The pacing functionality of the CardioECR system is suitable for both acute pacing regimens to evaluate compound effects on contractility under controlled beating rate and long-term stimulation used for improvement of functional maturation of hiPSC-CMs. For detailed information, please refer to our application note Using Agilent xCELLigence RTCA ePacer for Functional Maturation of human-induced Pluripotent Stem Cell-Derived Cardiomyocytes.

The CardioECR assay workflow consists of (Figure 2A):

1. Seeding hiPSC-CM into the wells of the CardioECR plate, and allowing contact with and attachment to the sensor-electrode surfaces.

2. The CardioECR plate is then placed inside the cradle and engaged within the CardioECR plate station, inside a tissue culture incubator.

The formation of a functional syncytium as evidenced by a robust and consistent contractile and electrophysiological recording can occur 4 to 14 days postseeding, depending on the cardiomyocyte (Figure 2B). Contraction and FP signals are detected via impedance electrodes and FP electrodes, respectively, and recorded by the CardioECR station. The data acquisition is controlled through RTCA CardioECR data acquisition software within the control unit. The offline CardioECR data analysis software provides in-depth data analysis functions to quantify both acute and long-term effects of compounds on the biological status of the cells, including cell viability, contractility, and electrophysiology.

A

B
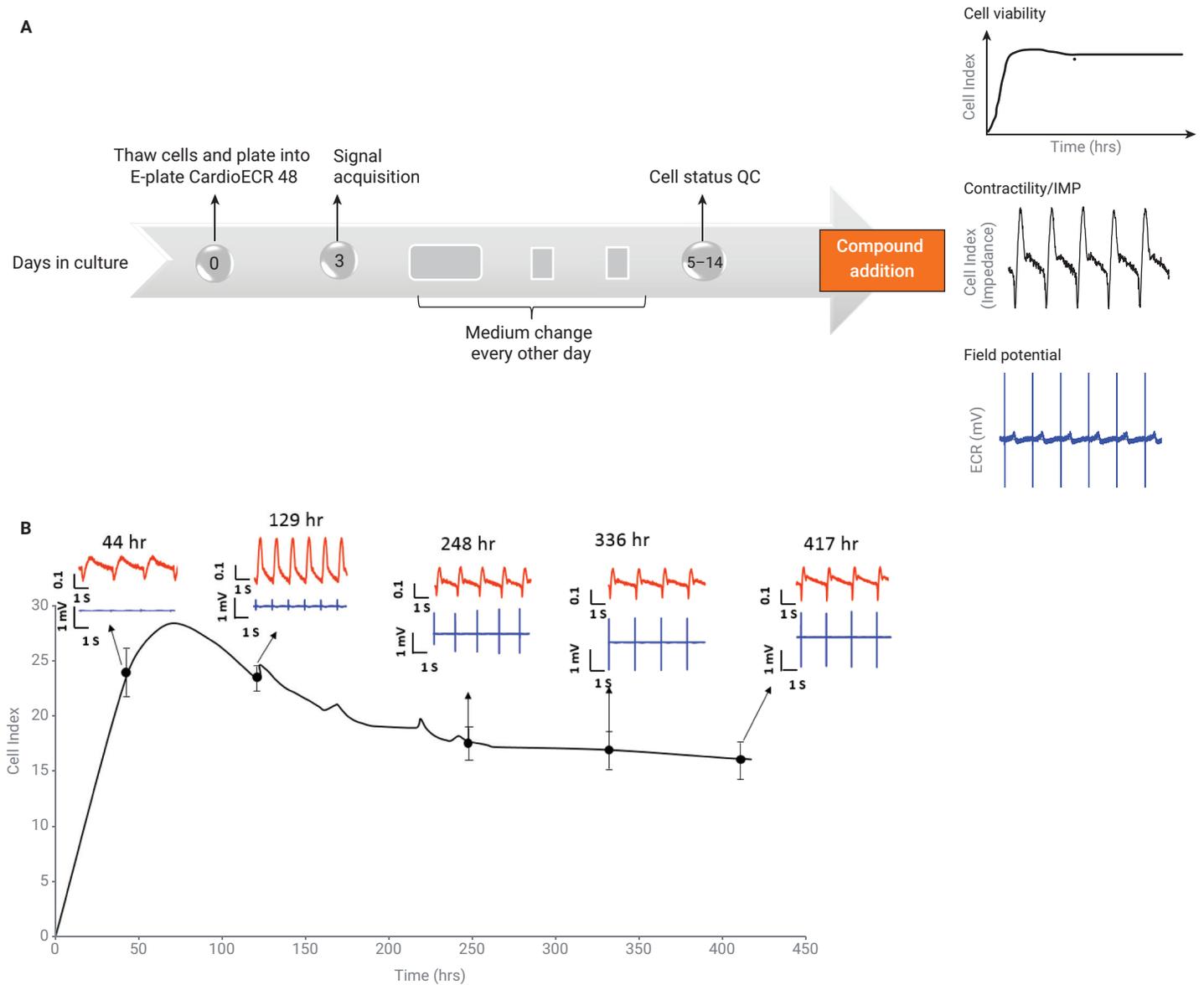
Figure 2. (A) The workflow of the hiPSC cardiomyocyte assay using the Agilent xCELLigence RTCA CardioECR system: cell seeding on day 0; start to measure cell performance on day 3; start cell status QC before compound addition; after treatment, cell viability, contraction, and electrophysiology are evaluated via IMP and FP readouts measured by the CardioECR system. (B) The overall Cell Index curve was recorded in real time after cell seeding. Cell contraction (red trace) and field potential signal (blue trace) were simultaneously measured along with overall Cell Index.

CardioECR software

Included with the Agilent CardioECR system are two software packages: the CardioECR data acquisition module and the CardioECR data analysis software. CardioECR data acquisition is mainly used for real-time signal measurement following the preprogrammed, user-defined experimental steps (Figure 3A). The predefined experiment schedule automatically controls the signal measurement during the experiment. In addition, it offers online data analysis functions for evaluation of cell contractility in real time as quantified by up to 13 parameters of IMP signal, such as beating rate (BR) and beating amplitude (BAmp). However, CardioECR data analysis must be used for more in-depth offline data analysis on both IMP and FP signals (Figure 3B). The software provides the user with more than 25 parameters based on IMP and FP signals to assess cardiac cell beating and electric activities. To analyze the complex wave forms derived from hiPSC-CMs contractility and FP responses, a number of parameters were derived. Contractility signal was evaluated using BR, beating period (BP), and BAmp parameters. BR is defined as the number of beats per unit of time and is expressed as beats/minute.
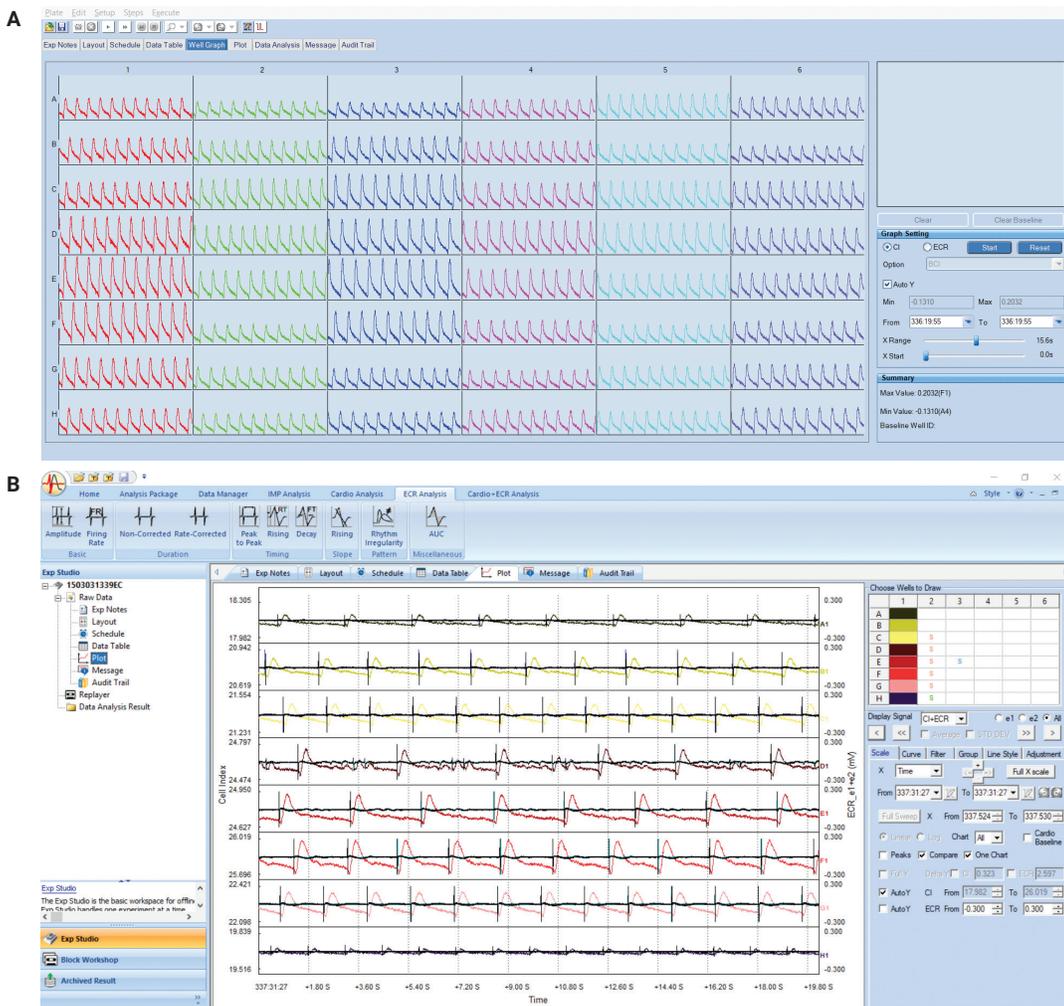

Figure 3A, B. (A) Screenshot of 15 second of impedance waveforms obtained from individual wells of an Agilent E-plate CardioECR 48 recorded by Agilent CardioECR data acquisition software. (B) Screenshot of Agilent CardioECR data analysis software: both IMP and FP traces were overlaid and displayed on the chart, which will be further analyzed using the data analysis functions provided in CardioECR data analysis software.

BP defines the time period between two successive major peaks. BAmp is defined as the absolute (delta) Cell Index (CI) value between lowest and highest points within a beating waveform (Figure 3C). For analysis of FP signal, the FP spike amplitude (FP-Amp) is derived, which is the absolute (delta) value in mV from the lowest point of the initial spike to the highest point of the spike. The FP duration (FPD) is defined as the time period between the negative peak of the FP spike to the maximum or minimum point of the reference wave. The reference wave can be negative or positive depending on how the cells are situated with respect to the FP electrodes (Figure 3C). The baseline performance of cardiomyocytes is an important parameter to determine if the cell being tested meets the minimal criteria for compound application. CardioECR data analysis provides an automatic function for cell status QC. As shown in Figure 3D, the cell contractile and electrical activities were evaluated by CardioECR data analysis software before compound addition.
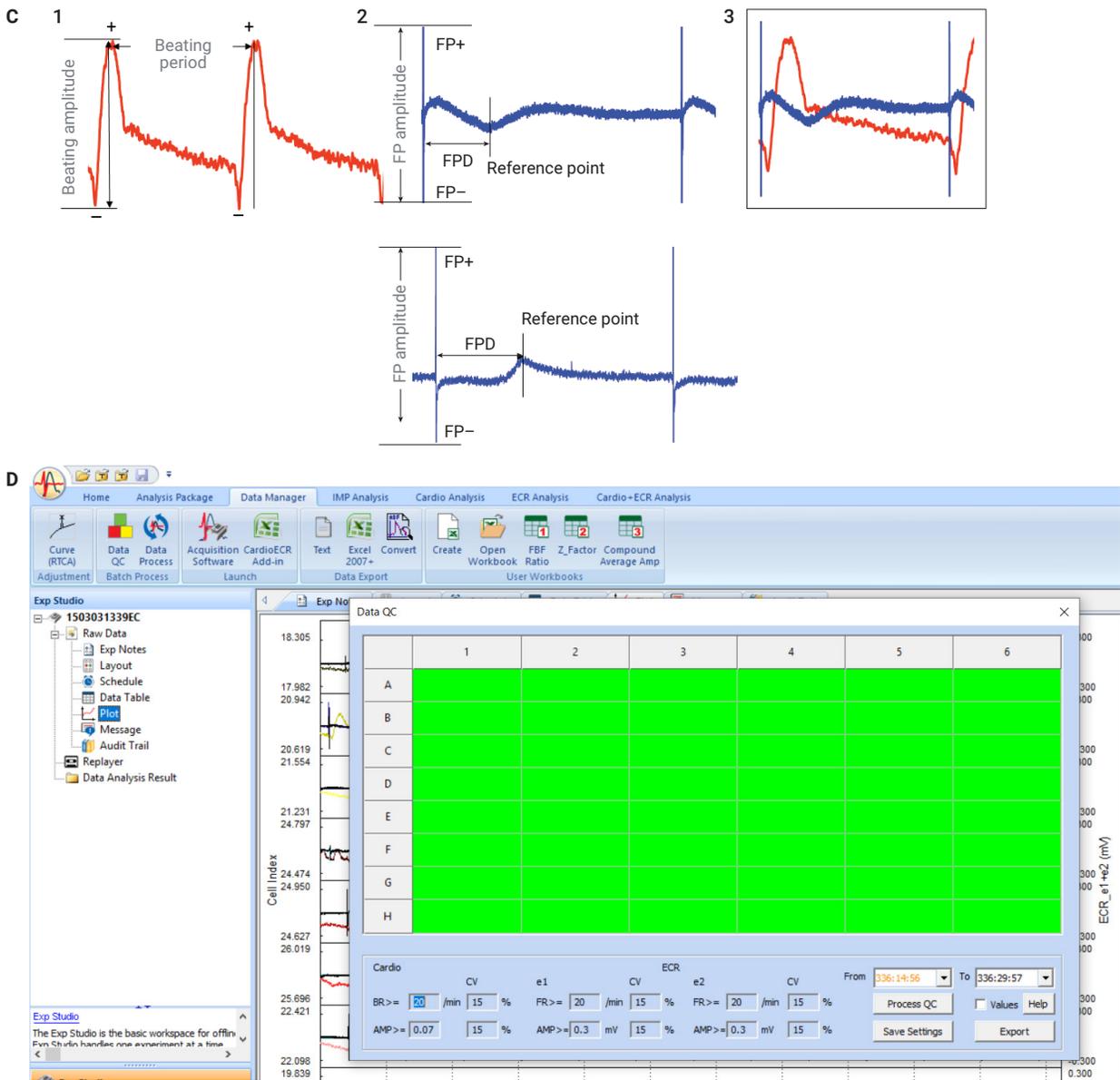
Figure 3C, D. (C) Definition of main parameters used to evaluate cell contractile and field potential activities. (1) The typical contraction pattern obtained from iCell CM2 and iCell CM. (2) The typical field potential (FP) waveforms obtained from iCell CM2 and iCell CM. The reference point can be negative (upper image) or positive (lower image). (3) Overlay of the IMP and FP traces. (D) Screenshot of the cell status QC function in CardioECR data analysis software: the green color of each well indicates that the cells in all the wells pass the QC and can be used for the compound test.

Experimental

Cell culture

HiPSC-CMs from FUJIFILM Cellular Dynamics International (FCDI/iCell Cardiomyocytes[2] (iCell CM2): R1017, or iCell cardiomyocyte (iCell CM): R1007; Madison, WI, USA) were stored in liquid nitrogen until they were thawed and cultured according to manufacturer instructions. Briefly, each well of the Agilent E-plate CardioECR 48 was coated with 50 µL of a 1:100 diluted fibronectin (FN) solution at 10 µg/mL (F1114, Sigma-Aldrich, St. Louis, MO, USA) and incubated at 37 °C for at least 1 hour, which was followed by replacing fibronectin solution with 50 µL of prewarmed iCell cardiomyocyte plating medium. Cells were thawed and diluted in prewarmed plating medium at the manufacturer-recommended concentration. A 50 µL amount of the cell suspension was transferred using a multichannel pipette and seeded directly onto a precoated E-plate CardioECR 48 (50,000 cells/well iCell Cardiomyocytes[2] (iCell CM2s); 20,000 platable cells for iCell cardiomyocytes (iCell CMs) in a laminar hood. The plates containing iCell CM2s or iCell CMs were kept in the hood at room temperature for 30 minutes, then placed and cultivated in a humidified incubator with 5% CO_2 at 37 °C. The plating medium was replaced with iCell cardiomyocyte maintenance medium 4 hours post-seeding for iCell CM2 and 48 hours post-seeding for iCell CM. Medium change was performed every other day afterwards.

Chemical reagents

All the chemical reagents were purchased from Tocris (Minneapolis, MN, USA), Sigma-Aldrich (St. Louis, MO, USA), or provided by the Chemotherapeutic Agents Repository of the National Cancer Institute. One-thousand-fold chemical stock solutions were prepared in DMSO and stored at −20 °C. The serial-diluted chemicals (1,000-fold) were further prepared in DMSO immediately before compound addition. The 10-fold final dilution of the chemicals was prepared with culture medium for single use only. The final concentration of DMSO in the treated well was 0.1%.

Results and discussion

Assessment of drug-induced proarrhythmia

Drug-induced arrhythmia and, in particular, drug-induced TdP, has been a major cause of clinical restrictions and market withdrawal.[6] A leading mechanism for drug-induced TdP is the prolongation of ventricular repolarization, termed the QT interval in an electrocardiogram (ECG), which may occur through inhibition of the hERG channel.[6] However, activity of other ion channels that regulate the action potential can influence hERG current block, and therefore hERG block alone sometimes does not provide a meaningful indication of QT prolongation. Furthermore, measurement of QT prolongation itself was shown to be highly sensitive for TdP, but not specific.[1,3,7,8] To address this limitation, a consortium sponsored by HESI and FDA, comprised of industry, academia, and government stakeholders, was formed under the CiPA initiative to come up with strategies, including using hiPSC-CM, for improving preclinical predictivity of arrhythmia.

The CiPA project was carried out in two phases: CiPA I and CiPA II. The xCELLigence RTCA CardioECR system was one of the platforms used for evaluation of drug-induced arrhythmia using hiPSC-CMs in all phases.[3] The data generated by the CiPA core sites and Agilent showed that the CardioECR system was sufficiently sensitive to identify torsadogenic compounds using different parameters including BAmp, BR, FPAmp, FPD elongation, and incidence of arrhythmic events such as EAD (Table 1).[9] In addition to using the changes in FPD and arrhythmic events using FP signal, drug-induced modulation of contractile responses via IMP readout was also investigated. As shown in Table 2, after 30 minutes of treatment with arrhythmogenic compounds, each compound induced irregular and asynchronous beating waveforms measured by IMP readout. The extent of arrhythmicity of IMP signal was further quantified by beating rhythm irregularity (BRI), a parameter representing the coefficient of variation of BP of each IMP waveform, which also showed drug-induced dose dependency. Interestingly, measurement of integrated ion channel activity by FP recording and contractility by IMP measurement displayed equal propensity to detect arrhythmia (Table 2). Overlay of the two signals shows very precise correlation in terms of detecting arrhythmic profiles such as ectopic beats as well as notch-like EADs. A recent study demonstrated that the CardioECR system was capable of distinguishing compounds with multichannel effects such as flecainide and quinidine.[9] Taken together, the integrated measurement of field potential and impedance measurement provided incisive information for prediction of drug-induced proarrhythmia.

Assessment of drug-induced cardiac contractile modulation

To investigate if the impedance signal represents contraction of hiPSC-CM, iCell CM2 were treated with the myosin heavy chain inhibitor, blebbistatin. It has been demonstrated that blebbistatin inhibits cardiomyocyte contraction with no significant impact on ion-channel activity leading to excitation-contraction decoupling.[10] As shown in Figure 4A, 0.4 µM blebbistatin induced a significant decrease in BAmp by 79 ±7%, and a moderate increase in BR by 10 ±6% in IMP readout. However, it showed subtle effects on field potential signal as shown by marginal changes in FPD and FP-Amp. The profound decrease in BAmp suggests that the IMP waveforms of beating cardiomyocytes are predominantly determined by physical movement or contraction.[5,11]

The relationship between amplitude of IMP waveform (BAmp) to the force of contraction was further investigated by applying nifedipine, an L-type Ca^{2+} channel inhibitor, to the hiPSC-CM seeded in the E-plates. Figure 4B shows that nifedipine caused a dose-dependent reduction in BAmp. The correlation between reduction in BAmp of IMP readout and decrease in contractility observed in the clinic after nifedipine treatment suggests that BAmp can be a surrogate for the force of contraction. Taken together, IMP readout of the CardioECR system provides a direct and quantitative readout of cardiomyocyte contractile activity, which is a very useful parameter for assessment of potential toxicities of pharmaceutical compounds.

Assessment of long-term effects of oncology drugs

The coexistence of cancer and cardiovascular diseases in the same patient is more common due to the aging population and improvements in the effectiveness of cancer drugs. Cardio-oncology, the multidisciplinary cardiovascular care of cancer patients,

Table 1. Summary of quantitative compound effects on electrophysiological and contractile activity of iCell CMs across three independent evaluation sites. The table is adapted from (Zhang, X., 2016, reference no. 9).
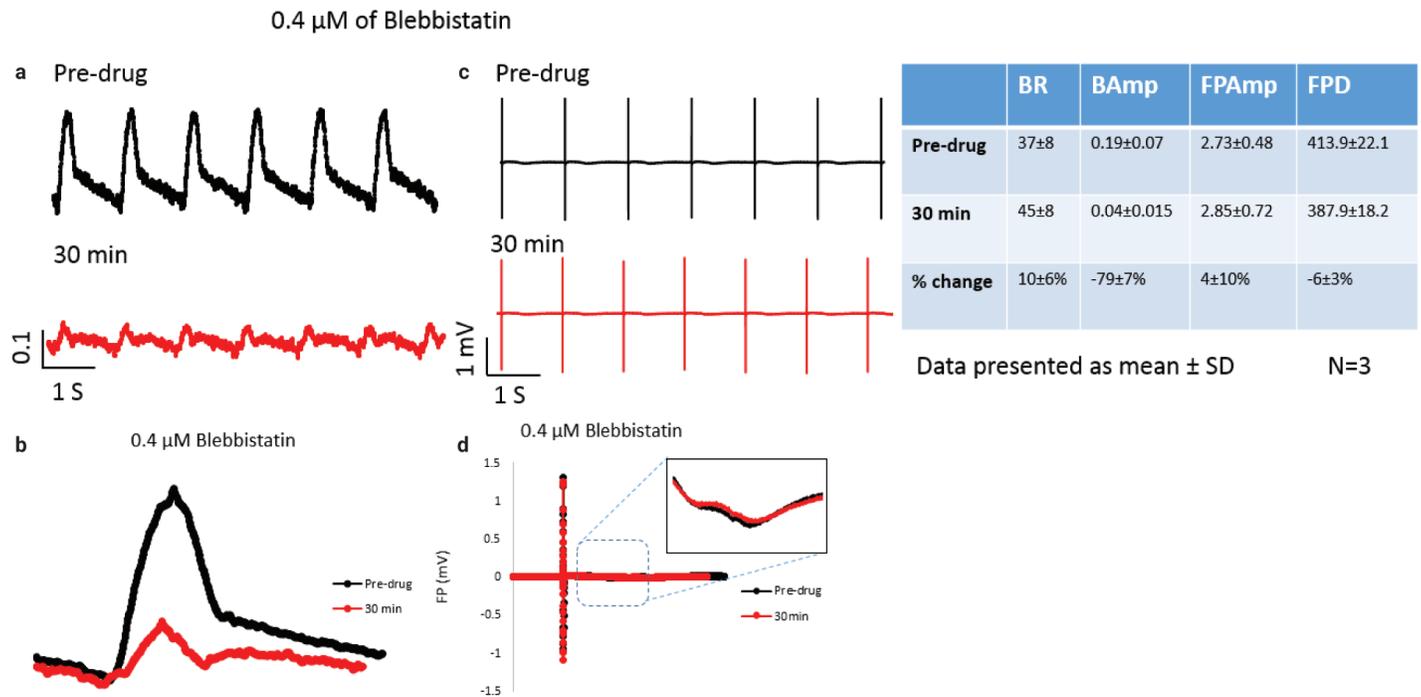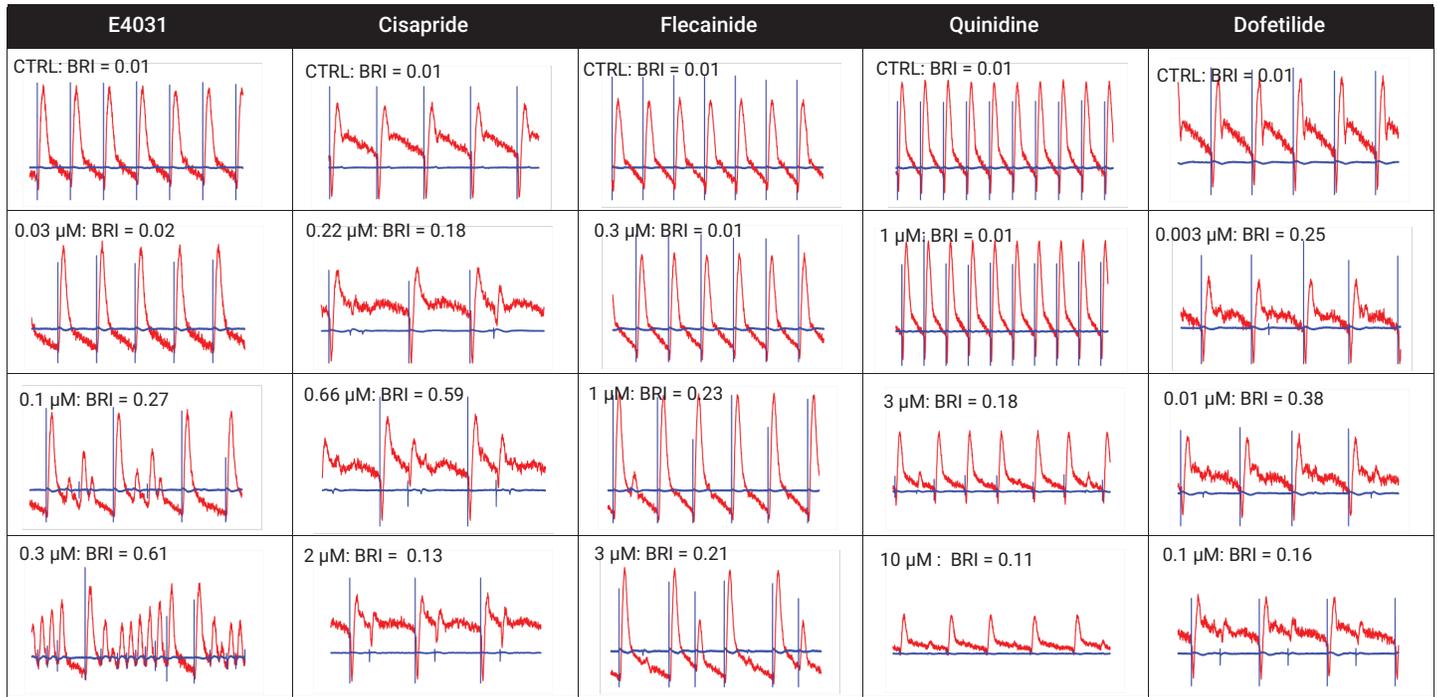
Compound	Conc. (µM)	% change of BAmp	% change of BR/FR	% change of FPAmp	% change of FPD	Arrhythmia/EAD
Flecainide	0.1	0±2%	3±6%	-6±10%	4±5%	No
	0.3	-2±2%	-6±14%	-28±14%	18±19%	Yes
	1	-3±1%	-27±22%	-46±31%	71±19%	Yes
	3	-20±2%	-48±13%	-81±14%	77±40%	Yes
Quinidine	0.3	-5±1%	-5±15%	-6±8%	4±9%	No
	1	-3±2%	-25±25%	-19±21%	22±23%	Yes
	3	-10±3%	-48±16%	-52±25%	35±39%	Yes
	10	-37±2%	-58±8%	-81±15%	12±32%	Yes
Nifedipine	0.01	-3±1%	17±9%	3 ± 9%	-11±9%	No
	0.03	-2±1%	31±18%	2 ± 14%	-18±14%	No
	0.1	-25±2%	66±19%	-6 ± 15%	-28±1%	No
	0.3	-62±5%	86±30%	-7 ± 11%	-40±4%	No
Moxifloxacin	3	-3±1%	4±7%	-24±35%	0±5%	No
	10	-3±3%	0±6%	-20±28%	8±10%	No
	30	-1±2%	-18±14%	-26±27%	44±23%	Yes
	100	-2±10%	-48±18%	-41±27%	80±60%	Yes
Mexiletine	1	0±2%	7±3%	-8±10%	-1±3%	No
	3	-2±2%	2±8%	-16±21%	3±6%	No
	10	-3±1%	-7±12%	-54±28%	20±21%	No
	30	-20±2%	-51±28%	-88±5%	N/A	Yes
JNJ303	0.01	-2±1%	8±3%	-5±7%	-1±3%	No
	0.03	-4±1%	7±3%	-5±15%	2±2%	No
	0.1	-3±1%	6±3%	-5±13%	13±4%	No
	0.3	-0±0%	6±4%	-9±9%	12±4%	No
Ranolazine	1	-4±2%	5±7%	-2 ± 9%	13±15%	No
	3	-3±1%	2±7%	2± 8%	34±30%	No
	10	-3±1%	-9±14%	-13± 16%	45±39%	Yes
	30	-8±6%	-20±10%	-23±14%	12±32%	Yes
E4031	0.003	-1±4%	-4±12%	-12±12%	34±14%	No
	0.01	-4±1%	-33±20%	-46±25%	N/A	Yes
	0.03	-32±9%	-65±8%	-71±24%	N/A	Yes
	0.1	-51±12%	-58±19%	-84±17%	N/A	Yes

has been proposed as a new approach to improve prevention, early identification, and management of cardiotoxicity. There is an urgent need to develop preclinical approaches, including cell-based assays using hiPSC-CM, to predict as early as possible potential cardiac toxicity induced by cancer drug candidates during the drug development phase.
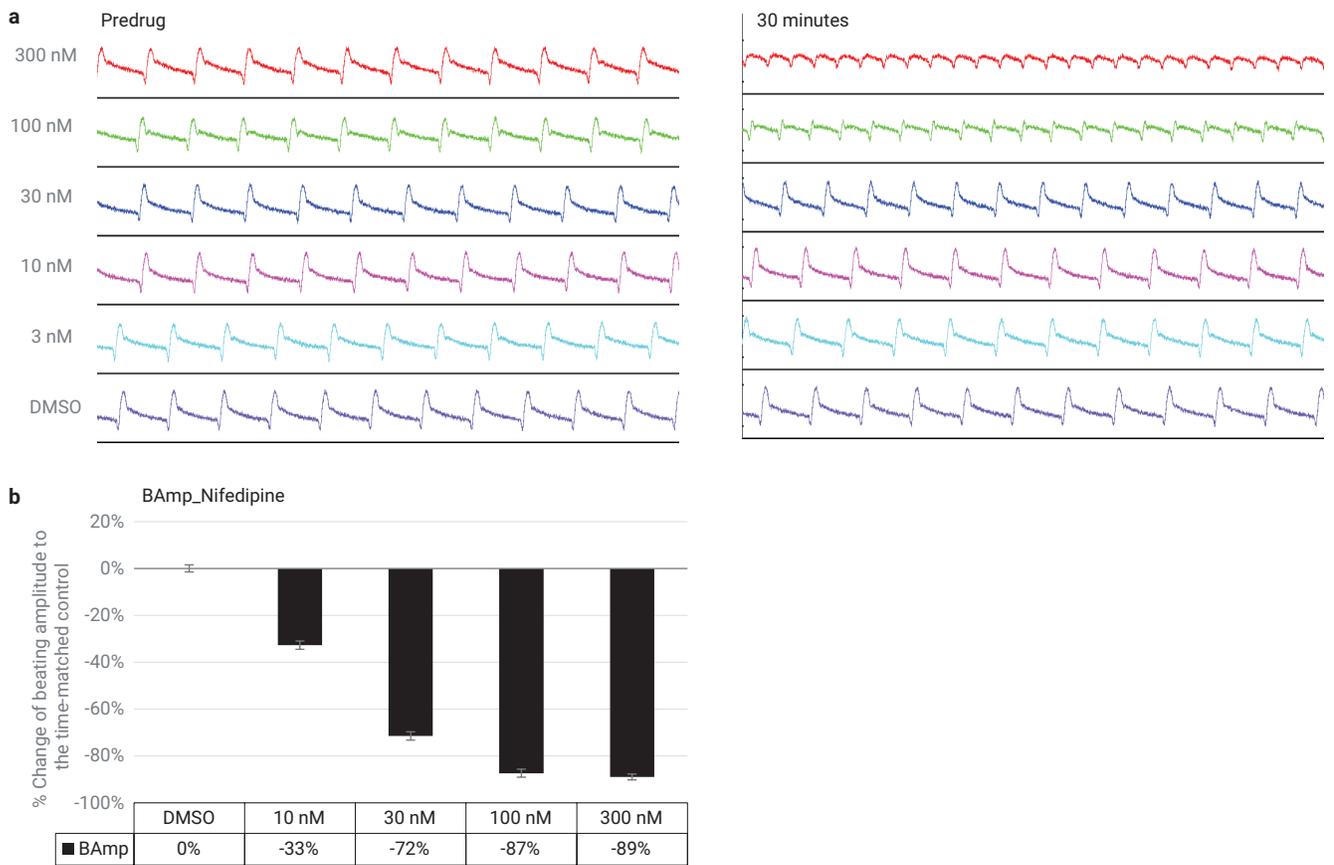
Most oncological drugs do not cause acute adverse side effects such as arrhythmia, but rather delayed onset of cardiotoxicity. To determine if hiPSC-CMs are capable of recapitulating long-term toxicity effects of oncological drugs in

the human heart, we treated hiPSC-CMs with doxorubicin. Doxorubicin is a chemotherapy drug that has been shown to induce chronic cardiotoxicity such as apoptosis, primarily due to oxidative stress, by interfering with mitochondrial function.[12] Using the CardioECR system, the drug was tested in iCell CMs 14 days postseeding, when the cells showed stable and robust beating and electrical profiles. Immediately after compound addition, the cell responses were continuously monitored and measured for up to 3 days.

Table 2. iCell CMs were treated with five proarrhythmogenic compounds. The IMP (red trace) and FP (blue trace) shown in the table were captured 30 minutes after compound addition. All traces shown are 10 seconds in duration. 0.1% DMSO serves as control. The compound-induced arrhythmias were followed at different concentrations. The table is adapted from (Zhang, X., 2016, reference no. 9).

E4031	Cisapride	Flecainide	Quinidine	Dofetilide
CTRL: BRI = 0.01	CTRL: BRI = 0.01	CTRL: BRI = 0.01	CTRL: BRI = 0.01	CTRL: BRI = 0.01
0.03 µM: BRI = 0.02	0.22 µM: BRI = 0.18	0.3 µM: BRI = 0.01	1 µM: BRI = 0.01	0.003 µM: BRI = 0.25
0.1 µM: BRI = 0.27	0.66 µM: BRI = 0.59	1 µM: BRI = 0.23	3 µM: BRI = 0.18	0.01 µM: BRI = 0.38
0.3 µM: BRI = 0.61	2 µM: BRI = 0.13	3 µM: BRI = 0.21	10 µM : BRI = 0.11	0.1 µM: BRI = 0.16

0.4 µM of Blebbistatin

	BR	BAmp	FPAmp	FPD
Pre-drug	37±8	0.19±0.07	2.73±0.48	413.9±22.1
30 min	45±8	0.04±0.015	2.85±0.72	387.9±18.2
% change	10±6%	-79±7%	4±10%	-6±3%

Data presented as mean ± SD   N=3

Figure 4A. iCell CM2 were treated with 0.4 µM blebbistatin. Six seconds of IMP waveforms measured before and 30 minutes postdrug (a). The averaged IMP waveform (b) calculated from (a), six seconds of FP waveforms measured before and 30 minutes postdrug. The averaged FP waveform (d) calculated from (c). These figures are adapted from Zhang, X. *et al.* 2016.[9]

a

Predrug | 30 minutes

300 nM
100 nM
30 nM
10 nM
3 nM
DMSO

b

BAmp_Nifedipine

% Change of beating amplitude to the time-matched control

|	DMSO	10 nM	30 nM	100 nM	300 nM
■ BAmp	0%	-33%	-72%	-87%	-89%

Figure 4B. iCell CM were treated with nifedipine. The 20 seconds of IMP traces were collected before and 30 minutes after drug addition, respectively (a). The bar graph of % change of BAmp after compound exposure to the time-matched DMSO control (b). The data were represented by mean ±SD, N = 3.

Figure 5A shows that the overall Cell Index curve significantly decreased in a time and dose-dependent manner, predominantly due to the loss of cardiomyocyte viability, which was confirmed by observation of cell death under a microscope. As shown in the inset of Figure 5A, at longer durations of doxorubicin exposure (56 hours), contractility of iCell CMs was also severely impaired. These drug-induced contractile changes may be associated with structural damage and significant cell loss, indicated by the decline in Cell Index value. The effects of doxorubicin on both cell contraction and electrical activity were further assessed and quantified by fold change in BR, BAmp, FP-Amp, and FPD postdrug. With the exception of subtle changes in FPD (data not shown), BR, BAmp, and FP-Amp all significantly decreased in a time and dose-dependent manner (Figure 5B). In summary, the multiplexing evaluation of chronic effects of compounds in hiPSC-CMs, including viability, contraction, and electrophysiology of cells from the same well and at the same time, allows an integrated analysis of compounds that may help unravel potential toxicity mechanisms of compounds.

In this application note, we evaluated acute and chronic effects of compounds using the xCELLigence RTCA CardioECR, which allows simultaneous measurement of IMP signal via IMP electrodes and FP signal via FP electrodes.

It has been shown previously that impedance measurement can monitor the spontaneous contraction of both primary cardiomyocytes and stem cell-derived cardiomyocytes,[13,14] as well as overall attachment and viability.[15] The xCELLigence RTCA CardioECR system can monitor the attachment and viability of hiPSC-CMs via IMP readout over the entire duration of the experiment in real time.[9] This is an appealing feature of the system, since it provides an internal built-in quality control for the cells, and any compounds that may cause structural damage or other types of toxicity can readily be detected, as it has been shown for some kinase inhibitors[7] and anthracyclines such as doxorubicin (Figure 5A). At high data acquisition of 500 Hz, the system
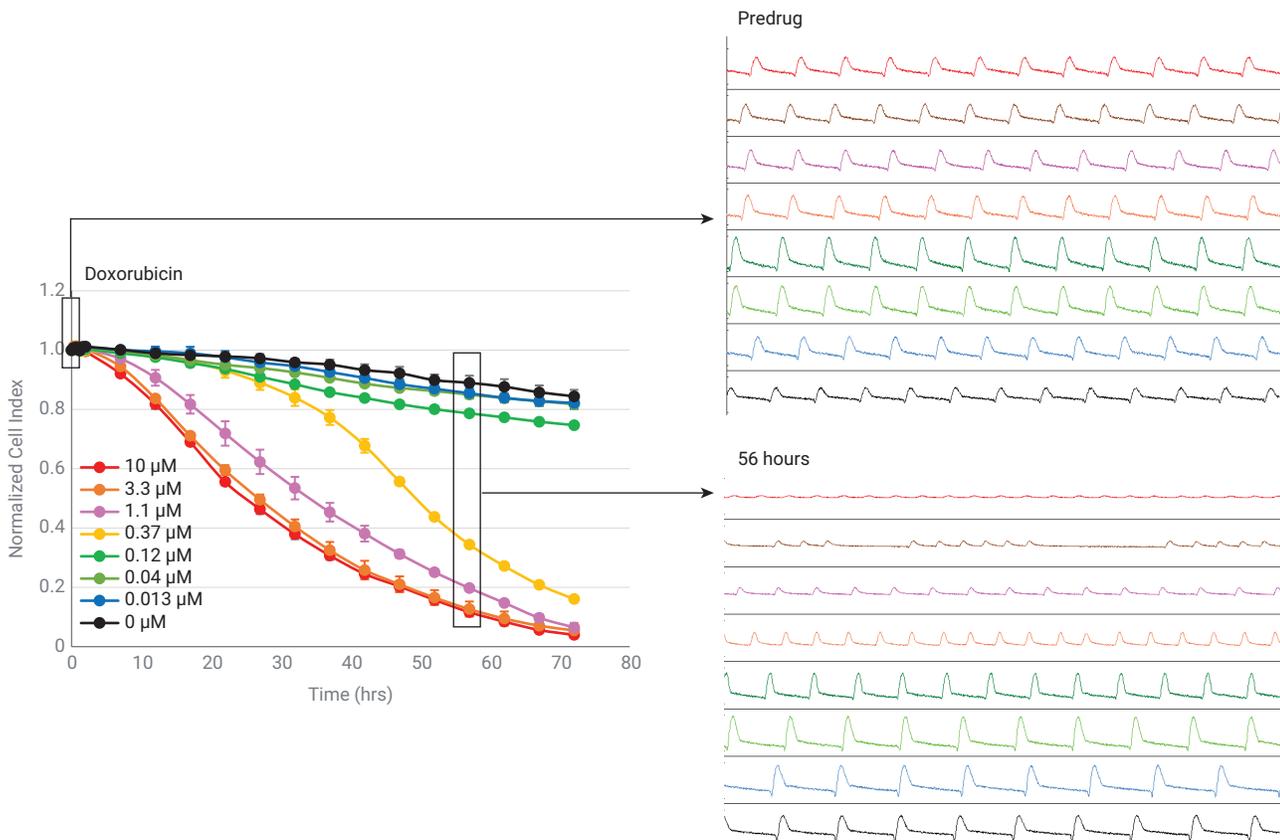
Figure 5A. Overall cell index change over the course of 72 hours treatment. Inset: 15 seconds of IMP traces obtained from iCell CM before and after doxorubicin treatment at the indicated time points.

BAmp

FP-Amp

BR
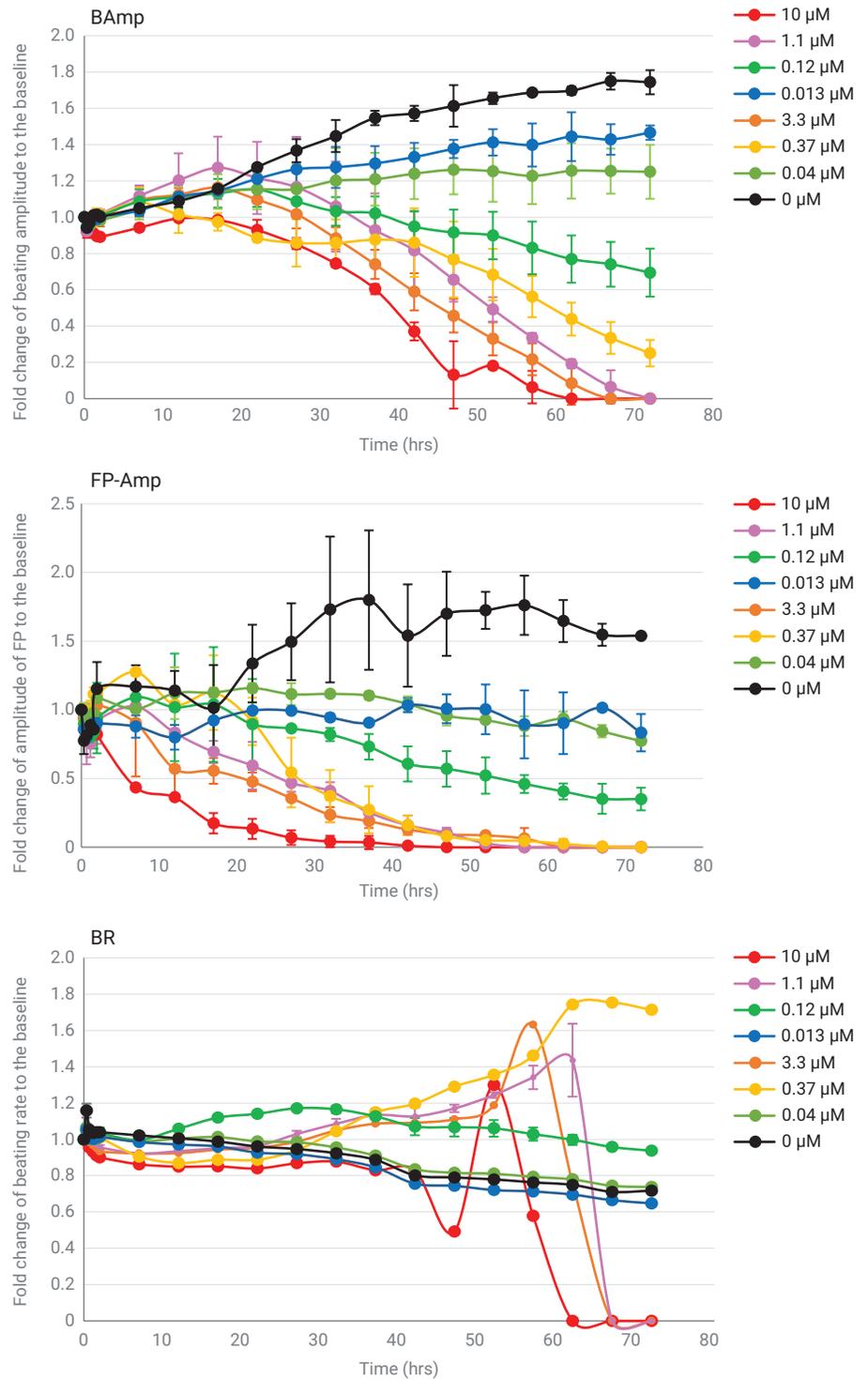
Figure 5B. The time course of fold change in BAmp, BR, and FP-Amp to the baseline over 72 hours treatment with doxorubicin. The data were represented by mean ±SD, N = 4.

can readily monitor the spontaneous beating of the hiPSC-CMs (the inset of Figure 5A). The periodic beating of the syncytia of hiPSC-CMs is highly sensitive to compounds that are known to modulate both the BR and extent of the contractions, and this syncytial periodic beating can be sufficiently sensitive, specific, and accurate.[5] In addition to contractility measurement, the FP readout of CardioECR can simultaneously monitor the integrated ion channel activity upon interaction with the membrane of the hiPSC-CMs (Figures 2B and 3B). The FP waveform can provide mechanistic information about how compounds can potentially affect various currents, as reported by Hallbach *et al.*[17] For example, the major current responsible for the FP spike is known to be the sodium current. FPD showed a direct relationship with action potential duration 90 (APD90) as measured by manual patch clamp, a critical parameter to assess cardiac repolarization.

The entire process of cardiomyocyte beating intricately links the integrated ion channel activity at the membrane to the release of Ca^{2+} from the sarcoplasmic reticulum, which in turn modulates the actin-myosin machinery to culminate in cardiomyocyte contraction.[16] From both a mechanistic and a safety/toxicity perspective, it is imperative to assess the effect of compounds simultaneously on all of these events. We and others[9-11] have shown that the compound blebbistatin, an inhibitor of myosin heavy-chain ATPase, can decouple excitation from contraction without any detectable effect on the FP recording but with profound effect on contractility. The data support the premise that measuring both aspects of excitation and contraction is pertinent to obtaining a more comprehensive assessment of compound effect on hiPSC-CMs.

Conclusion

We showed that the assessment of potential cardiac toxicities of new chemical/biological entities in beating cardiomyocytes, which require the excitation-contraction coupling mechanisms to fulfill their functional activity, necessitates the simultaneous and long-term combined measurement of FP and IMP parameters, facilitated with electrical pacing when it is needed via the use of the xCELLigence RTCA CardioECR system.

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