Application Note

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Connected Workflow: Assessment of Inotropic Compounds in Paced Cardiomyocytes Using Calcium Imaging

Authors

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Abstract

This application note demonstrates the feasibility of using a connected workflow to evaluate the effect of inotropic compounds on electrically paced human iPSC-derived cardiomyocytes (hiPSC-CMs) through calcium imaging. The workflow is instituted using the electrical pacing feature of the Agilent xCELLigence RTCA ePacer to achieve functional maturation of hiPSC cardiomyocytes. Once functional maturity of the cells has been achieved, based on a positive force-frequency relationship (FFR), inotropic compounds are added to the culture medium. The cellular responses to inotropes are monitored by fast-kinetic measurement of Ca^{2+} transient on the Hamamatsu FDSS/µCELL system. Our data demonstrates that the workflow provides higher translatable results to assess the modulation of cardiomyocyte function by inotropic compounds. It uses a physiologically relevant readout for screening of efficacious inotropes in addition to assessment of potential liability of pharmaceutical drugs.

Introduction

The heart is one of the key organs that is susceptible to adverse drug effects.[1] The potential of drug-induced life-threatening polymorphic ventricular tachyarrhythmia, known as torsades de pointes (TdP), has increasingly resulted in termination of drug candidates during the late preclinical or clinical stages and issuance of warning or withdrawal of numerous drugs from the market.[2] However, drug-induced cardiac inotropic changes, including both decreased and increased contractility, can also lead to cardiovascular risks (CV).[3] Negative inotropes may cause heart failure, while positive inotropes may elicit an increase in myocardial oxygen demand, associated with a risk of myocardial hypoxia and increased mortality. Evidence has shown that several drug classes are associated with changes in the left ventricular ejection fraction (LVEF), a clinical output reflecting cardiac contractility, leading to altered therapeutic use, label warnings, and drug withdrawal from the market.[3]

Current approaches to assessing cardiomyocyte contractility include the Langendorff assay, using isolated animal hearts and echocardiography using conscious telemetrized animals, such as dogs and rats. They have been used to predict drug-induced inotropic effects in humans.[4,5] However, the drawbacks of these methods, including labor-intensive procedures, high cost, and low throughput, limit their utility in early drug discovery. Also, the requirement for cross-species translation complicates the process of assessing human safety risks. To identify and mitigate the risks of changing contractility during early drug development, a low-cost and mid-to-high-throughput screening assay using a physiologically relevant cell model is highly desired.

Numerous studies have demonstrated the ability of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) as model systems to detect electrophysiological and contractile effects of drugs, such as drug-induced prolongation of repolarization[6,7] and alteration of contraction.[8] Despite these promising results, many lines of evidence indicate that under the current standard cell culture conditions, instead of displaying morphological and functional characteristics of adult cardiomyocytes, hiPSC-CMs resemble embryonic cardiomyocytes.[9-11]

The immature phenotype of hiPSC-CMs has hindered full use of the cardiomyocytes for cardiac drug discovery, especially inotropic compounds, due to lack of distinction between negative and positive inotropic effects.[12] Numerous *in vitro* approaches, including long-term cell culture, growth factor stimulation, physical or mechanical stimulation, substrate stiffness, and electrical stimulation, have been described to improve the maturation status of hiPSC-CMs.[13]

Current *in vitro* approaches to evaluate contractile liability of drugs are primarily focused on using hiPSC-CMs, which are cultivated under standard cell culture conditions. This application note describes an innovative approach to promote maturation of hiPSC-CMs before compound addition.

As shown in Figure 1, when cells generate stable and robust beating activity (approximately seven days postseeding), a continuous electrical pacing procedure is applied to the cells for 15 days using the Agilent xCELLigence RTCA ePacer system (RTCA ePacer). Immediately before compound treatment, a simple and quick assessment of force-frequency relationship (FFR) of paced cells is performed on the RTCA ePacer. If the cells show an inversion of FFR from negative to positive, which reflects improved maturation status, compound treatment will then begin. The Ca^{2+} transients before and up to 30 minutes after compound addition are measured directly from the E-Plates containing the paced cardiomyocytes using the Hamamatsu FDSS/µCELL system to assess inotropic effects of drugs.

Experimental

Materials and methods

Cell culture

iCell Cardiomyocytes^2 (iCell CM^2), human iPSC-derived cardiomyocytes, were purchased from FUJIFILM Cellular Dynamics International (FCDI) (R1017, Madison, WI, U.S.). The cells were stored in liquid nitrogen until they were thawed and cultured according to the manufacturer's instructions. Briefly, each well of the E-Plate Cardio View 96 (300601080, Agilent Technologies, San Diego, CA, U.S.) was coated with 50 µL of a 1:100 diluted fibronectin (FN) solution at 10 µg/mL (F1114, Sigma-Aldrich, St. Louis, MO, U.S.) and incubated at 37 °C for at least 1 hour. The fibronectin solution was then replaced with 50 µL of prewarmed iCell cardiomyocyte plating medium.

Cells were thawed and diluted in the prewarmed plating medium at 1,000,000 cells/mL. FIfty microliters of the cell suspension was transferred using a multichannel pipette and seeded directly onto a precoated E-plate Cardio View 96 (50,000 cells/well) in a laminar hood. The plates containing iCell CM^2 were kept in the hood at room temperature for 30 minutes and then cultivated in a humidified incubator with 5% CO_2 at 37 °C. The plating medium was replaced with iCell cardiomyocyte maintenance medium 4 hours postseeding. A medium change was performed every other day afterward.

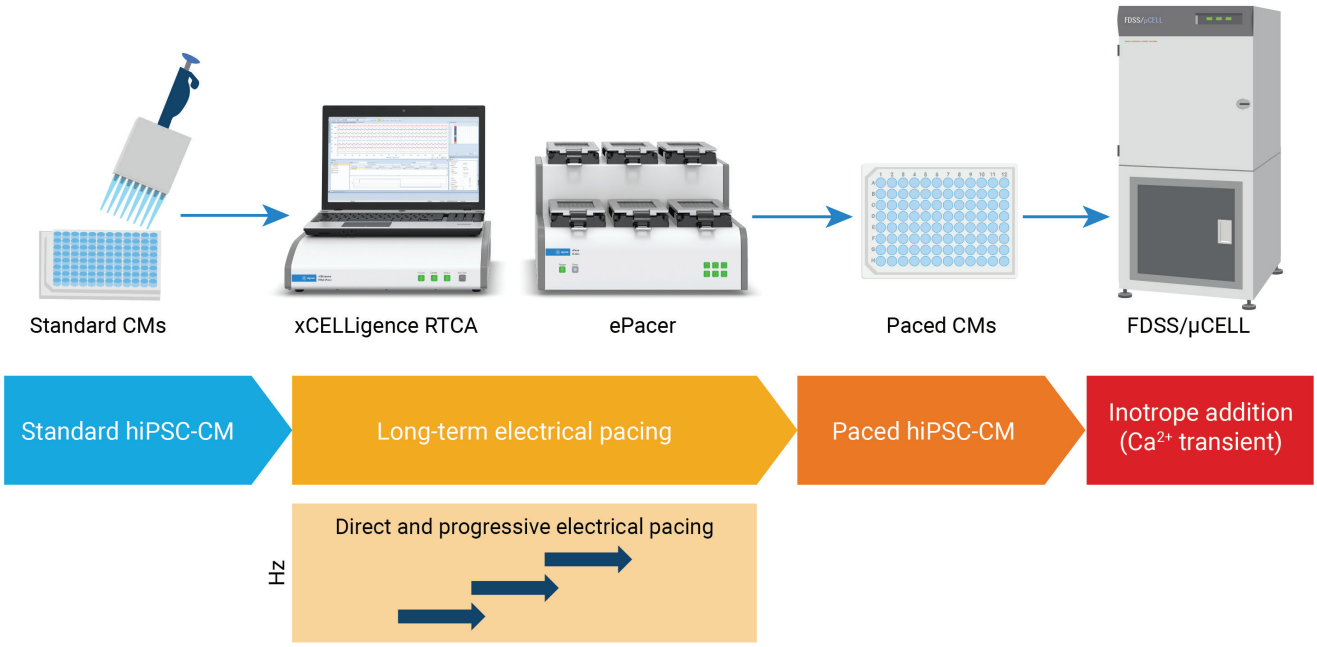


Figure 1. The workflow of inotrope screening in chronically paced hiPSC-derived cardiomyocytes (hiPSC-CMs). hiPSC-CMs were seeded directly onto the Agilent E-Plate Cardio View 96. Attachment, viability, and functional activity of cells were monitored and recorded on the Agilent RTCA ePacer after cell seeding. Once the cells generated stable and consistent functional activity (day seven, postseeding), they were subject to a directed, progressive electrical pacing, which was followed by an assessment of functional maturation on the RTCA ePacer. The cells were treated with inotropes if they met the assay criteria. The inotropic effects of drugs were evaluated by Ca^{2+} transients that were recorded directly from the E-Plate Cardio View 96 on the FDSS/µCELL system.

Functional maturation of hiPSC cardiomyocytes using RTCA ePacer

Following seven days of iCell CM[2] culture, after establishing consistent and robust contractile activity as measured by impedance, electrical stimulation was continuously applied to the cells and progressively increased at fixed intervals. The cells were subjected to a pacing frequency starting at 1 Hz, followed by 1.5 Hz, and ending at 2 Hz. The duration of each applied pacing frequency lasted for five days. The stimulus setting mainly depends on cell status and properties, which could vary. The entire pacing process takes approximately two weeks.

Alternatively, a four-day maturation protocol can also be used seven days postseeding. The cells are subjected to electrical stimulation at 1 Hz for one day, followed by consecutive three-day pacing at 2 Hz.

Assessment of maturation status of long-term paced hiPSC cardiomyocytes on RTCA ePacer

The day before compound addition, the maturation status of paced cells was assessed using FFR as an indicator on the RTCA ePacer system. The pacing was stopped for 6 hours before FFR test, which ensured the full recovery of cell spontaneous beating activity during the 6-hour break.

The hiPSC-CMs were then subjected to electrical pacing with increasing frequencies (0.75, 1, 1.5, and 2 Hz). The stimulus setting for the highest pacing frequency (2 Hz in this case) was optimized before the FFR test started. The beating rate and beating amplitude were only recorded after the cells had been paced for 10 minutes at each pacing frequency on the RTCA ePacer. After the test, the FFR calculation function on the RTCA ePacer software was used to generate a linear curve between average beating rate and average beating amplitude of all paced wells.

Evaluation of cardiac contraction using Ca^{2+} transients on FDSS/µCELL

Following 15 days of pacing, Ca^{2+} transients in hiPSC-CMs, cultured in E-Plates Cardio View 96 were assessed using EarlyTox Cardiotoxicity Kit (R8210, Molecular Devices, San Jose, CA, U.S.). The FDSS/µCELL system (C13299, Hamamatsu, Japan) combined with the Ca^{2+}-sensitive fluorescent dye (Cardiotox) can be used to monitor changes in intracellular Ca^{2+} concentrations associated with cardiomyocyte contraction. Briefly, the spent cell maintenance media were replaced with 90 µL of 1:1 mixture of Cardiotox and maintenance medium (dye solution) 4 hours after termination of pacing. The cells were preincubated with the

dye solution in a humidified incubator with 5% CO_2 at 37 °C for 2 hours before drug addition.

In the meantime, the temperature of the plate stage of the FDSS/µCELL system was set up at 37 °C. After a measurement of the baseline of Ca^{2+} flux for 60 seconds on the FDSS/µCELL system, the cell plates were then transferred to a laminar hood for compound addition. Ten microliters of 10x compound solutions were added to each well. After 30 minutes of compound treatment in the incubator, the plates were transferred to the FDSS/µCELL system for another 60 seconds measurement of Ca^{2+} transient.

Chemical reagents

Isoproterenol (1747), milrinone (1504), BayK 8644 (1546), and isradipine (2004) were purchased from Tocris ((Minneapolis, MN, U.S.). Omecamtiv mecarbil (S2623) was purchased from Selleck Chemicals (Houston, TX, U.S.). 1,000-fold compound stock solutions were prepared in DMSO and stored at –20 °C. The serial-diluted compounds (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 wells was 0.1%.

RTCA ePacer and assay principle

The Agilent xCELLigence RTCA ePacer is a dual-mode instrument that includes real-time monitoring of hiPSC-CM viability and contraction, as well as directed electrical pacing of hiPSC-CMs. It consists of a control unit (computer and software), an ePacer analyzer, an ePacer station that is permanently placed inside a CO_2 incubator, and E-Plates, which are specialized electronic microplates (Figure 2A) placed within the cradles of the ePacer. Embedded within the bottom of the E-Plate wells are interdigitated gold microelectrodes and impedance (IMP) electrodes (Figure 2B), which noninvasively monitor cell impedance signal, providing a

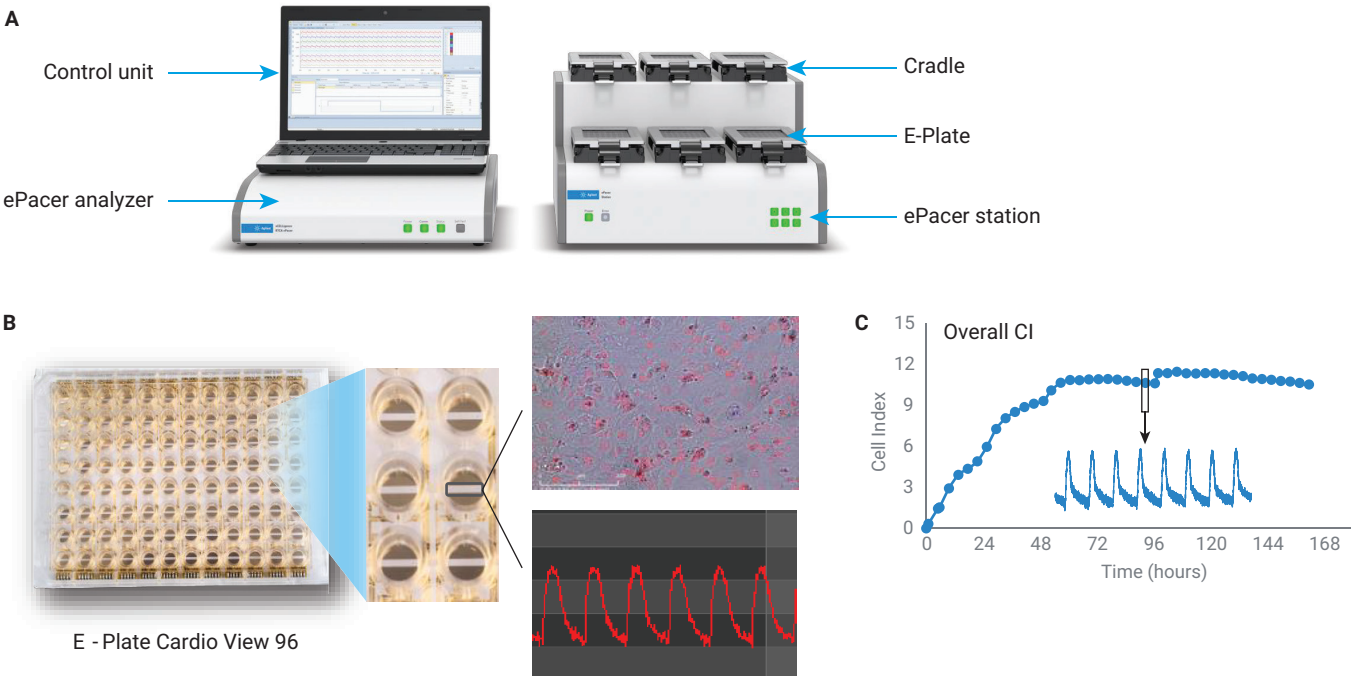


Figure 2. The Agilent xCELLigence RTCA ePacer system. (A) The system consists of four components: Control unit (laptop), ePacer analyzer, ePacer station, and E-Plates. (B) An image of the Agilent E-Plate Cardio View 96. On the E-Plate Cardio View 96, a close-up of the wells reveals the layout of the electrodes as well as the "view area", an electrode-free gap that enables users to observe the cardiomyocytes in the wells using brightfield and fluorescence channels. The upper image shows a superimposed image of brightfield and red fluorescent staining of nuclei of hiPSC-CMs. The lower image shows raw traces of fluorescence-labeled Ca^{2+} transient measured on the FDSS/µCELL system. (C) Typical data obtained from an RTCA ePacer system, including the overall Cell Index curve for cell viability assessment and high-frequency data acquisition of Cell Indexes, which form waveforms for contraction evaluation.

measure of hiPSC-CM viability and, under high-frequency data acquisition mode, a measure of contractility (Figure 2C). The cellular impedance signal is recorded based on user-defined time intervals (minutes and hours) and is reported using a unitless parameter called Cell Index.

During the electrical pacing, the electrical pulses are directly applied to the cells through the embedded IMP electrodes at the bottom of the wells. For most cardiomyocytes, the length of each electrical pulse applied through the IMP electrode is less than 4 milliseconds, which allows the contractile activities of cells to be immediately captured and recorded while the cells are being paced via IMP electrodes. The exact conditions for electrical pacing are dependent on the cell type, the inherent beating frequency, and the experimental context. This workflow application used an E-Plate Cardio View 96 (96-well plate) due to its compatibility with the Hamamatsu FDSS/µCELL system and the "view area" that allows imaging of the cells (Figure 2B).

Besides Cell Index for assessment of cell viability and health, the ePacer software provides the user with basic contractile parameters, beating amplitude, and beating rate. These parameters are extracted and saved after each measurement. Beating rate is defined as the number of beats per unit of time and is expressed as beats/minute. Beating amplitude is defined as the absolute (delta) Cell Index (CI) value between the lowest and highest points within a beating waveform. In addition, the force-frequency relationship function in the software eases the process of revealing the relationship between beating amplitude, a surrogate of force of contraction, and beating rate.

FDSS/µCELL and assay principle

The FDSS/µCELL system is a kinetic plate reader that includes a dispensing head and optics for fluorescence and luminescence detection (Figure 3). The system can simultaneously measure the kinetics of the fluorescence or luminescence intensity from 96 or 384-well microplates without a time lag. The beating activity of cardiomyocytes is very sensitive to temperature, especially the spontaneous beating rate, which easily decreases at temperatures lower than 37 °C. The heater unit underneath the assay plate stage maintains a 37 °C temperature to ensure stable beating and proper responses of cardiomyocytes to treatment. The high-speed data acquisition can acquire images with short interval times (10 milliseconds) to accurately measure the calcium oscillation in cardiomyocytes. The FDSS/µCELL Waveform Analyzer software allows quick and easy analysis of the waveform of calcium oscillation, including waveform amplitude (Ca_Amp), beating rate, area under the curve, and up to 16 other parameters.

Figure 3. The Hamamatsu FDSS/ µCELL system is a kinetic plate reader, which includes dispensing head and optics for fluorescence and luminescence detection. (need feedback from Hamamatsu)

Results and discussion

Chronic electrical pacing improves contractile maturity of hiPSC cardiomyocytes

To achieve enhanced predictivity for drug-induced inotropic effects using hiPSC-CMs, functionally matured cardiomyocytes were prepared using long-term electrical pacing before the application of inotrope compounds. When stable and robust beating activity (7 days postseeding) was achieved, the cells were subjected to chronic electrical pacing, increasing the pacing frequency from 1 Hz (5 days) to 1.5 Hz (5 days) and finally 2 Hz (5 days). To determine if the cells had gained enhanced functional maturity from the pacing procedure, the relationship between beating rate and beating amplitude as FFR, which is a validated surrogate for force of contraction, was evaluated.[8]

The FFR test was performed on the day before compound addition (14 days postpacing). Since chronic pacing causes temporary cessation of beating activity, electrical pacing was paused for 6 hours before the FFR test to allow the cells to recover their spontaneous functional activity at approximately 30 beats/min. Then, a short stimulation pulse (10 minutes for each pacing frequency) was applied to the cells to control and increase the beating rate. The beating amplitude was measured after cells established a stable beating activity at different beating rates (45, 60, 90, and 120 beats/min) using the RTCA ePacer. Figure 4 shows the beating amplitude and beating rate relationship that was achieved before and after chronic pacing on the RTCA ePacer. It demonstrates that the inherent negative FFR of hiPSC-CMs reversed to a positive relationship, suggesting that the cells gained a higher degree of functional maturity via the long-term electrical pacing.

Measurement of Ca^{2+} transient in functionally matured hiPSC cardiomyocytes

The day before compound addition, FFR of chronically paced iCell CMs was evaluated. If the cells passed the assay criteria, showing positive FFR, the compound treatment would be performed the next day. After the FFR assessment, the electrical pacing of the hiPSC-CM was resumed until 4 hours before adding the cardiotox dye solution to the cells. The cells were preincubated with the dye solution in the incubator for 2 hours and closely monitored and evaluated on the RTCA ePacer via an impedance readout to ensure their spontaneous beating activity, as assessed by beating rate and beating amplitude, was fully recovered before compound addition.

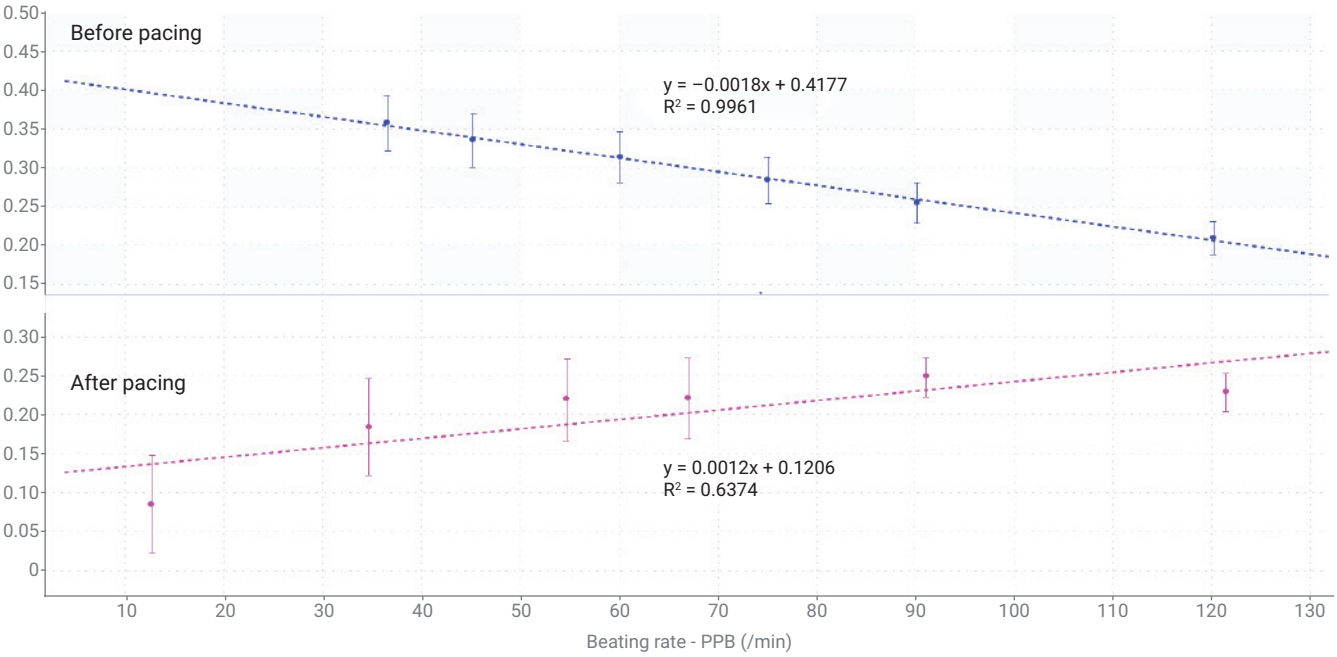
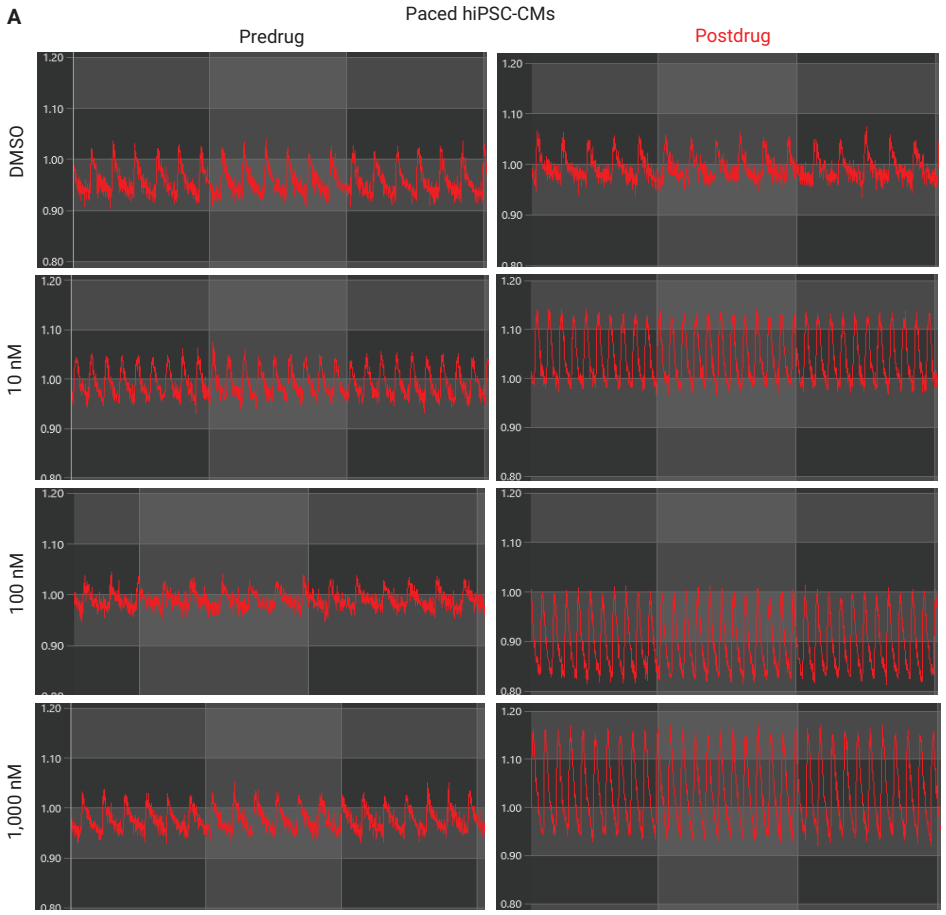
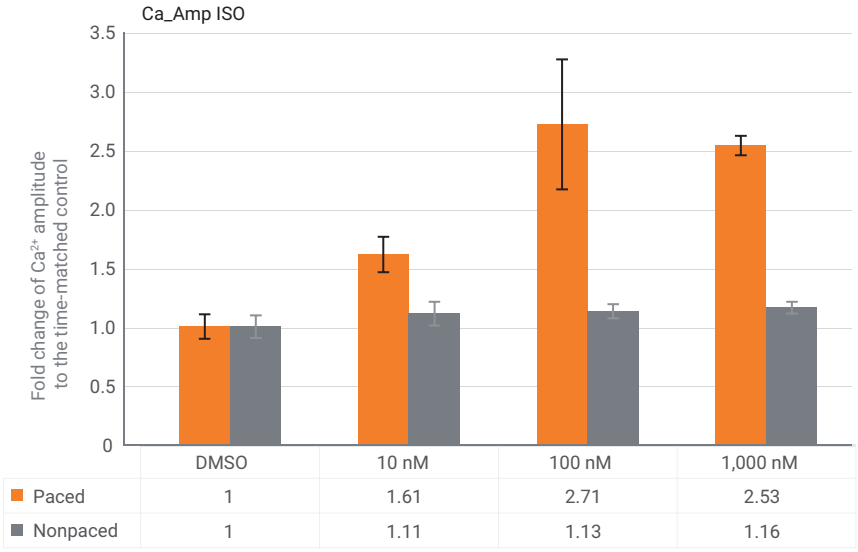


Figure 4. The relationship between beating amplitude/impedance amplitude and beating rate evaluated before (blue trace) and after (pink trace) chronic electrical pacing was applied to hiPSC-CMs. The data were represented by mean ±SD, N = 48.

The Ca^{2+} transient before and 30 minutes after drug treatment were measured on the FDSS/µCELL system for 60 seconds at 20 frames/s and 37 °C. To demonstrate that functional maturation of hiPSC-CMs is pivotal for revealing appropriate pharmacological responses to inotropes, nonpaced cells in the experiment as the control group were also included. While FDSS/µCELL software provides up to 16 parameters based on Ca^{2+} transient waveforms, this application note only focused on the changes in amplitude of Ca^{2+} transient (Ca_Amp), which reflects the force of contraction.

A 0.2-fold difference from the baseline was used as "significant", independent of test concentration, because this magnitude of effect exceeds baseline variability. The first test was β-adrenergic receptor agonist isoproterenol (ISO), a well-known positive inotropic and chronotropic compound, at multiple concentrations. ISO increases cAMP and renders an increase in intracellular Ca^{2+} concentration via the PKA pathway, which ultimately increases cardiac contraction. As shown in Figure 5A, ISO significantly increased Ca_Amp and showed a clear dose-dependency only in electrically paced hiPSC-CMs (paced cells). The nonpaced cells only had marginal responses to ISO and displayed the maximal increase by 0.15-fold at 1,000 nM ISO. Milrinone, a phosphodiesterase-3 inhibitor that increases intracellular cAMP, gave the same profile as ISO. The profound increase in Ca_Amp was only observed in the paced cells. BayK 8644 increases contractility via activation of the L-type Ca^{2+} channel. The remarkable escalation of amplitude in Ca^{2+} transient was detected in both paced and nonpaced hiPSC-CMs after BayK 8644 addition (Figure 5C). However, the magnitude of increase in paced cells was much larger than that of the nonpaced cells (1.41-fold compared to 0.23-fold). The





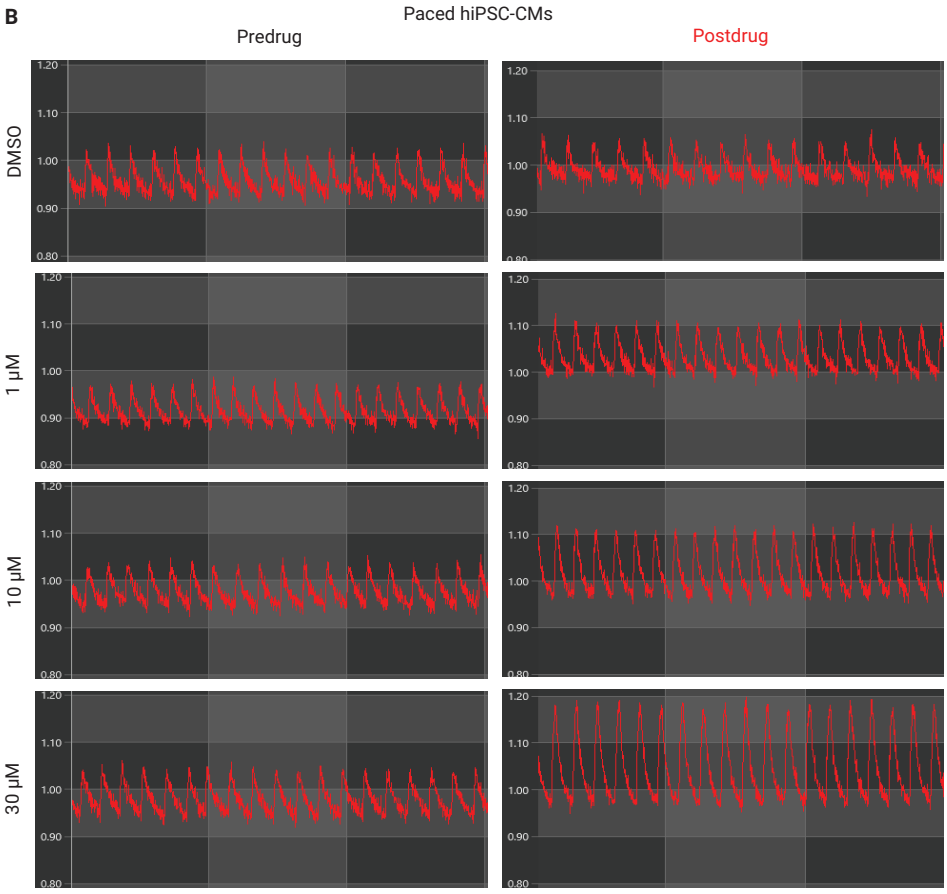
	DMSO	10 nM	100 nM	1,000 nM
Paced	1	1.61	2.71	2.53
Nonpaced	1	1.11	1.13	1.16

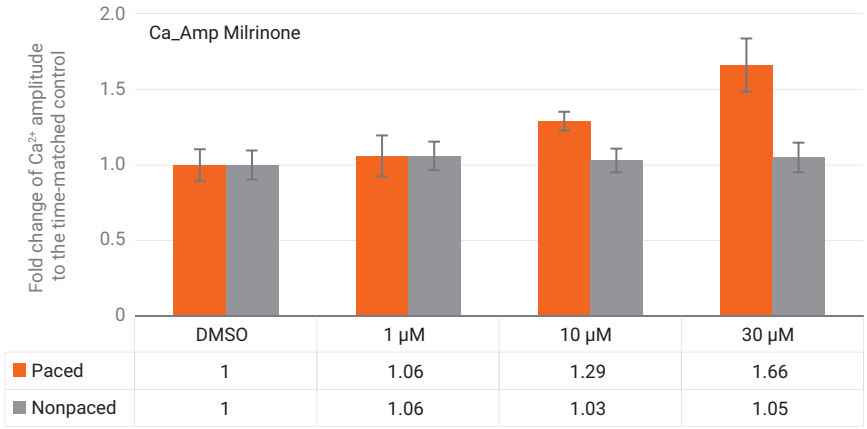
Figure 5. Inotrope-induced changes in Ca^{2+} transient. (A) The representative traces of Ca^{2+} flux in the paced cells before and 30 minutes after the addition of ISO at 10, 100, and 1,000 nM, which was further quantified (orange bars) along with the data collected from nonpaced cells (gray bars). The fold-change of Ca^{2+} transient amplitude to the time-matched DMSO control is shown 30 minutes after exposure to (B) milrinone at 1, 10, and 30 µM, (C) 300 nM BayK 8644, and (D) isradipine at 30 and 300 nM. The orange bars represent the data obtained from chronic paced cells. The gray bars are the results obtained from nonpaced cells. The data were represented by mean ±SD, N = 4.

L-type Ca^{2+} channel inhibitor isradipine reduced Ca_Amp and displayed the same level of reduction in paced cells as in nonpaced cells (Figure 5D). These data demonstrate that electrical pacing leading to functional maturation of hiPSC-CM can display the appropriate Ca^{2+} transient in response to positive inotropic compounds.

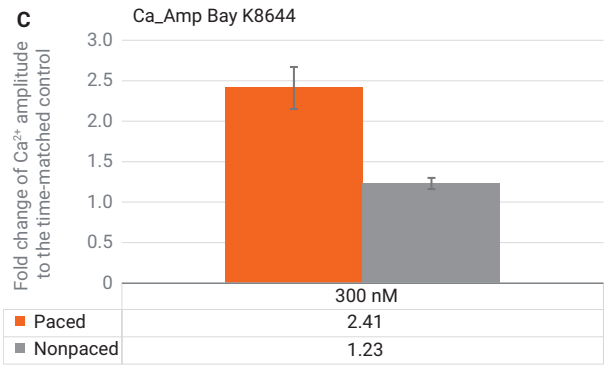
Amplitude of Ca^{2+} transient used for evaluation of cardiac contraction

As changes in intracellular Ca^{2+} concentration are intrinsically linked to changes in cardiomyocyte contraction, this study investigated if Ca^{2+} transient could be a suitable readout for the evaluation of cardiac contraction. Agilent has validated what other researchers have also found that high-speed impedance measurements reflect cardiac contractility and the amplitude of impedance waveform (IMP_Amp)/ beating amplitude can be used as a surrogate for the force of contraction.[8,16] The results of an impedance-based assay were compared to the results of Ca^{2+} transient after exposing cells to a panel of inotropes. The impedance assay was performed on the RTCA ePacer in parallel with a Ca^{2+} flux assay. To ensure that the assays were performed under similar experimental conditions, half of the same E-Plate was subject to impedance measurements, while the other half was used for Ca^{2+} transient measurements. These two readouts were measured on the RTCA ePacer or FDSS/µCELL sequentially.

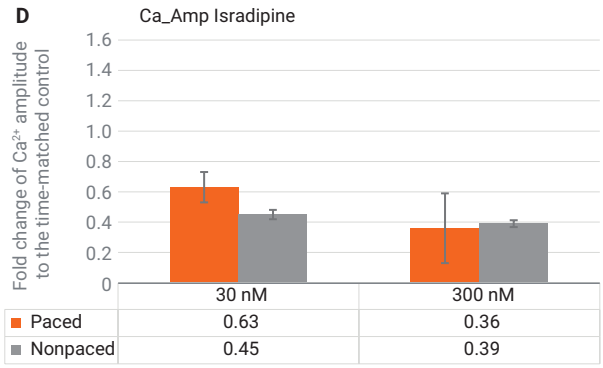




	DMSO	1 µM	10 µM	30 µM
Paced	1	1.06	1.29	1.66
Nonpaced	1	1.06	1.03	1.05

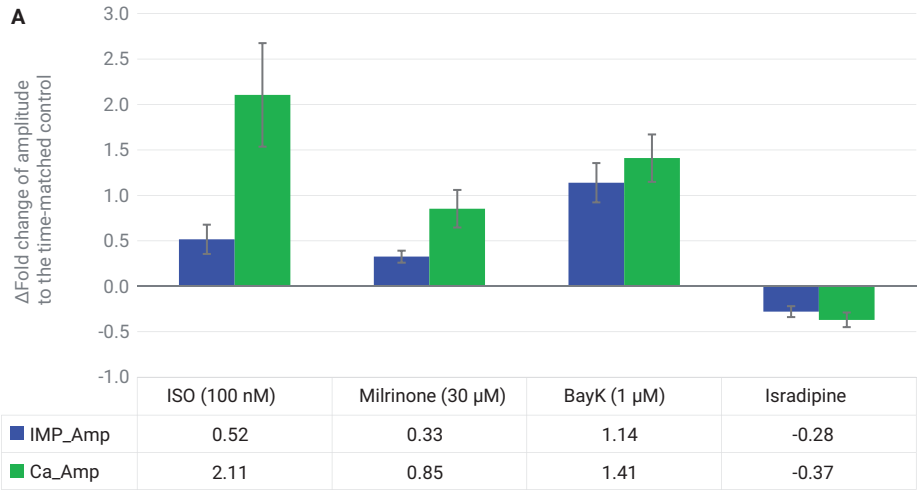


	300 nM
Paced	2.41
Nonpaced	1.23

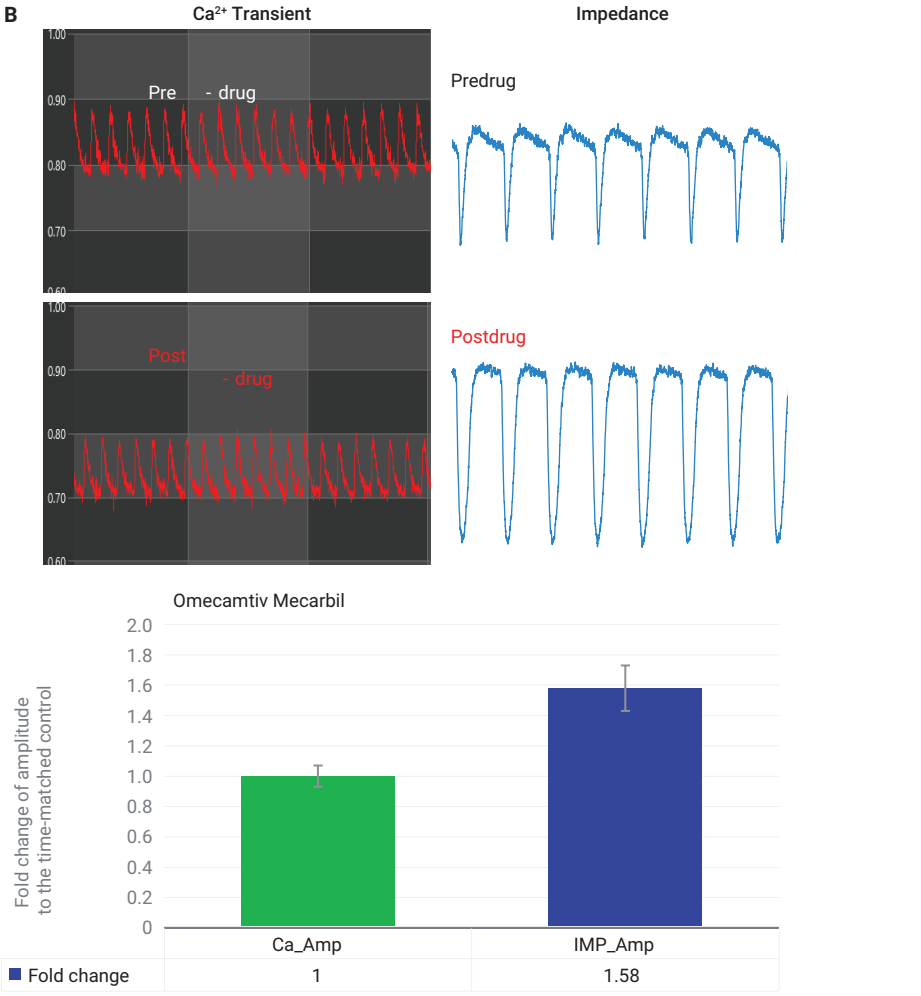


	30 nM	300 nM
Paced	0.63	0.36
Nonpaced	0.45	0.39

In this study, the cells were also prepared using electrical pacing for 15-days before compound addition. The fold changes of IMP_Amp and Ca_Amp shown in Figure 6A indicate that all the tested inotropes, no matter whether they had a negative or positive impact on contractility *in vivo*, had similar effects on IMP_Amp and Ca_Amp. This occurred despite the fact that drug-induced alteration of Ca^{2+} transient was more potent than impedance responses. The direction of changes in both IMP_Amp and Ca_Amp was consistent with the pharmacological mechanisms of the compounds. These data demonstrate that Ca^{2+} flux can be used as a surrogate for cardiac contraction in functionally matured hiPSC-CMs when evaluating compounds that affect the generation of calcium transient. Interestingly, inotropes with calcium-independent mechanisms, such as cardiac myosin activator, will not be detected using this method. As shown in Figure 6B, omecamtiv mecarbil, a myosin activator, profoundly increased IMP_Amp while showing no impact on Ca_Amp, even in electrically paced hiPSC-CMs.



	ISO (100 nM)	Milrinone (30 µM)	BayK (1 µM)	Isradipine
IMP_Amp	0.52	0.33	1.14	-0.28
Ca_Amp	2.11	0.85	1.41	-0.37



	Ca_Amp	IMP_Amp
Fold change	1	1.58

Figure 6. The inotropic effects of drugs in chronically paced hiPSC-CMs. (A) The delta fold-change of impedance amplitude (blue bars) and Ca^{2+} transient amplitude (green bars) at 30 minutes postdrug. (B) Representative traces showing a recording of Ca^{2+} transient on the FDSS/µCELL system and a recording of impedance waveforms on the RTCA ePacer system before and 30 minutes after omecamtiv mecarbil addition. The fold-change of Ca^{2+} transient amplitude (green bar) and impedance amplitude (blue bar) to the time-matched DMSO CTRL are shown at 30 minutes postdrug. The data were represented by mean ±SD, N = 4.

Conclusion

hiPSC-CMs are derived from reprogrammed somatic cells. Numerous lines of evidence indicate that these cells structurally resemble embryonic CMs and lack the complex structure and signaling mechanism required for efficient excitation-contraction coupling.[13] In the adult cardiomyocytes, isoproterenol displays positive inotropy and positive chronotropy, while hiPSC-CMs exert positive chronotropy but no positive inotropy, suggesting that the excitation-contraction machinery has not fully developed in these cardiomyocytes.[8]

To unleash the full potential of hiPSC-CMs as a physiologically relevant model for translational medicine, preclinical *in vitro* drug toxicity, and pharmacological analysis, various *in vitro* approaches have been developed for improvement of maturity of hiPSC-CMs. Electrical stimulation is one approach. This study has demonstrated that chronic pacing can improve functional maturity of hiPSC-CMs in our previous study.[17] So, for this application note, cells were electrically paced for 15 days on the RTCA ePacer to achieve functionally mature hiPSC-CMs before compound screening. The data showed that the inherent negative FFR of hiPSC-CMs reversed to positive FFR after 15 days of cell preparation (Figure 4). Since a positive FFR is a functional attribute of mature cardiomyocytes, the data shown here demonstrates that long-term electrical pacing can improve the functional maturity of hiPSC-CMs. While there are several ways to evaluate the maturation status of hiPSC-CMs, the direction of FFR can be used as a simple index for functional maturity. The automatic FFR evaluation feature of the RTCA ePacer software provides users with a quick and easy way to determine if the cells are functionally ready for the follow-up compound screening.

Even though several types of E-Plate can be used on the RTCA ePacer, this study used the E-Plate Cardio View 96 due to its compatibility with both the RTCA ePacer and FDSS/µCELL system. When the electrical pacing regimen was completed on the RTCA ePacer, the plates containing the functionally matured cells were transferred directly to the FDSS/µCELL system for the measurement of Ca^{2+} transient. It should be noted that the E-Plate Cardio View 96 is also compatible with other orthogonal readouts, such as high-content imaging and voltage-sensitive dye imaging.

The improved *in vitro* to *in vivo* pharmacological correlation observed in chronically paced hiPSC-CMs compared with nonpaced hiPSC-CMs was evident across a range of different positive inotropic drugs targeting a diverse set of signal pathways. Our Ca^{2+} transient data elicited that ISO, β-adrenergic receptor agonist, and milrinone, the phosphodiesterase-3 inhibitor, only significantly increased amplitude of Ca^{2+} transient in paced cells, but not in nonpaced cells (Figure 5A, B). The L-type Ca^{2+} channel activator, BayK 8644, also showed a much more profound increase in Ca_Amp in paced cells compared to nonpaced cells (Figure 5C). Interestingly, different responses to negative inotropic compounds, such as isradipine, were not observed between paced and nonpaced cells (Figure 5D). These data indicate that long-term pacing improves responses of hiPSC-CMs to positive inotropes, while having a very subtle impact on negative inotropic phenotypes. Also, the enhanced maturity developed in the long-term paced hiPSC-CMs enables them to distinguish positive inotropes from negative inotropes via the direction of changes in the amplitude of Ca^{2+} transient. However, this can't be achieved in standard/nonpaced cells.[14]

To determine if alterations of Ca^{2+} transient amplitude can represent drug-induced inotropic effects, Ca^{2+} transient data were compared to the results obtained from the impedance readout. The impedance amplitude and calcium transient amplitude correlated very well in paced cardiomyocytes for those inotropic compounds which mechanistically act through modulation of calcium from internal stores (Figure 6A). However, for the inotropes that directly target myofibrils, such as omecamtiv mecarbil (Figure 6B), their inotropic effects can only be detected by the impedance readout. This suggests that the measurement of Ca^{2+} transient amplitude is a suitable parameter for assessment of cardiac contraction when evaluating inotropic compounds that act through modulation of calcium. In comparison, an impedance-based contractility assay can measure inotropic compounds that act through calcium-dependent and independent pathways. The combined inotropic profile obtained from these two measurements provides confirmative results and mechanistic insights into compounds in terms of potentially targeted pathways in the excitation-contraction coupling process.

In summary, this application note demonstrates a workflow that combines two different platforms, the RTCA ePacer and FDSS/µCELL system, to assess drug-induced contractile effects on hiPSC-CMs. The RTCA ePacer is positioned as the first step and uses a long-term pacing procedure to produce a more physiologically relevant model than the standard hiPSC-CMs that are cultured under regular cell culture conditions. The cell preparation on the RTCA ePacer ensures higher predictive results of inotropic effects from drugs generated by the follow-up measurement of Ca^{2+} transient on FDSS/µCELL system. The workflow also offers insight into the mechanism of cardiomyocyte contraction.

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