VALA
SCIENCES

Simultaneous detection of action potentials and contractility in human stem cell-derived cardiomyocytes

INTRODUCTION

Cardiotoxicity—a drug side effect that impairs heart function—is a major cause of drug withdrawal, even after extensive cardiosafety screening and market launch.[1,2] Cardiotoxic drugs may target cardiac ion channels and interfere with the transient membrane depolarizations (action potentials) that maintain regular heart beats.[3] Many of these drugs inhibit potassium flux through the Kv11.1 channel (aka the human Ether-à-go-go-Related Gene, or hERG), which increases action potential duration and the risk of arrhythmia or sudden cardiac death (Figure 1).[4] Other cardiotoxic drugs damage structural and force-generating heart proteins, causing heart muscle to weaken and/or enlarge and leading to cardiomyopathy or heart failure.[5]

Current preclinical cardiosafety testing systems include non-cardiac cell lines engineered to express cardiac ion channels, isolated rabbit or canine heart muscle cells (cardiomyocytes), and toxicity studies in laboratory animals.[6,7] These systems are time-consuming, expensive, and incompletely model the electrophysiology and genetic variation of human cardiomyocytes. Many testing paradigms rely on one or a few parameters (e.g., action potential duration) and likely miss factors needed to detect genuine cardiotoxicity. By contrast, cardiomyocytes differentiated from human stem cells (hSC-CMs) exhibit the functional contractile phenotype of human ventricular cardiomyocytes, making them ideal for developing high-throughput, multi-parametric assays that predict cardiotoxicity with high sensitivity and specificity.[8,9]

In this pilot study, we aimed to develop a single assay that measures hSC-CM action potentials and contractility. We used Vala's IC200 Kinetic Image Cytometer® (an automated, high throughput digital microscopy workstation designed to record images at high frame rates) paired with Vala's CyteSeer® cell image analysis software to detect cell contraction with fluorescent membrane probes and transient membrane depolarizations with FluoVolt™ (a small
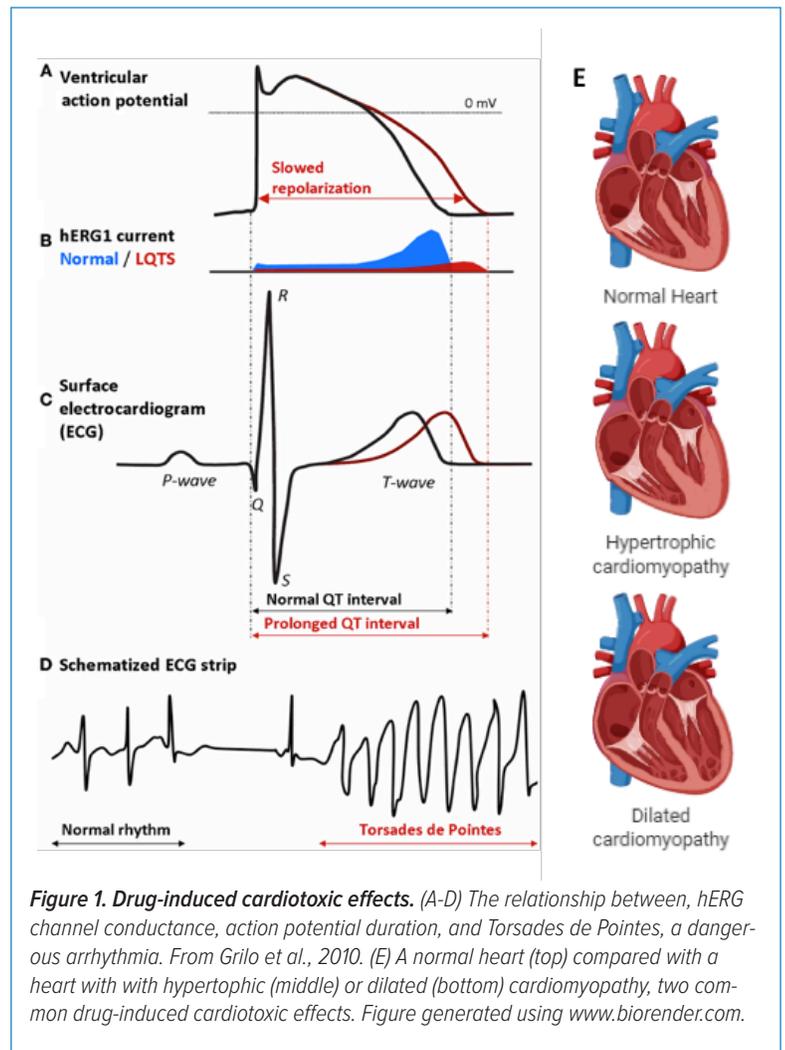
Figure 1. Drug-induced cardiotoxic effects. *(A-D) The relationship between, hERG channel conductance, action potential duration, and Torsades de Pointes, a dangerous arrhythmia. From Grilo et al., 2010. (E) A normal heart (top) compared with a heart with with hypertophic (middle) or dilated (bottom) cardiomyopathy, two common drug-induced cardiotoxic effects. Figure generated using www.biorender.com.*

molecule fluorescent voltage probe).[10] Using this assay, we tested two compounds with known effects on cardiac action potentials: dofetilide, which prolongs action potentials by inhibiting potassium flux through hERG, and nifedipine, which shortens action potentials by inhibiting calcium flux through L-type calcium channels. Our results demonstrate that Kinetic Image Cytometry® can simultaneously collect action potential and contractility data from hundreds of hSC-CMs, laying the groundwork for high-throughput, accurate, and cost-effective drug cardiosafety screening.
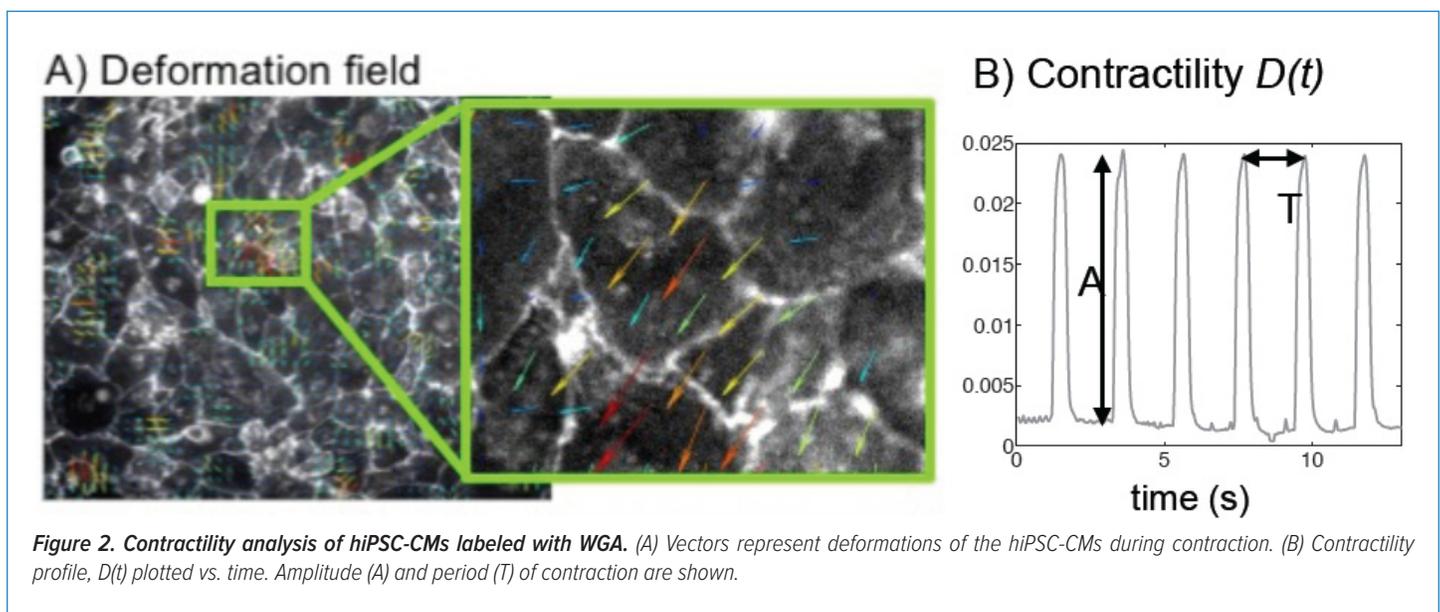
METHODS

Initial experiments using KIC® methods to quantify contractile motion (Figure 2) were carried out with human induced pluripotent stem cells differentiated to a ventricular cardiomyocyte phenotype (hiPSC-CMs) by researchers from the laboratories of Juan Carlos del Álamo (University of California, San Diego) and Mark Mercola (Stanford University). For these experiments, cells were labeled with 5 µg/mL wheat germ agglutinin (WGA) conjugated to AlexaFluor-488, which binds to the plasma membrane and enables KIC® to quantify cell contraction.

To simultaneously quantify contractile motion and voltage transients (action potentials), hSC-CMs (NCardia, Cor.4U Cardiomyocytes) were used. hSC-CMs were cultured in accordance with protocols suggested by the cell manufacturer in 96-well plates featuring optically clear well bottoms (Cat. No. 655090, Greiner, Monroe, NC). Cells were maintained at 37°C and 5% CO_2 for 6 days prior to imaging in culture media from the cell manufacturer. The cells displayed spontaneous beating within 48 hours of thawing.

The cells were loaded for 30 minutes at 37°C with FluoVolt™ as per the manufacturer instructions. The loading buffer contained 20 mM HEPES in HBSS and 200 ng/mL Hoechst 33342 (a live-cell nuclear stain). The cells were rinsed and then treated with DMSO alone, 10 nM dofetilide, or 1µM nifedipine (final DMSO concentration: 0.1%) for 20 minutes prior to imaging. Both drug incubation and imaging were done at 37°C. Since FluoVolt™ localizes to the plasma membrane, its fluorescence can be used to track membrane movement during contraction, similar to cells labeled with WGA-AlexaFluor-488. Vala researchers developed image analysis algorithms for KIC® that can simultaneously quantify movement of FluoVolt™-labeled cell membranes and voltage-dependent changes in FluoVolt™ intensity of the cells in the field of view.
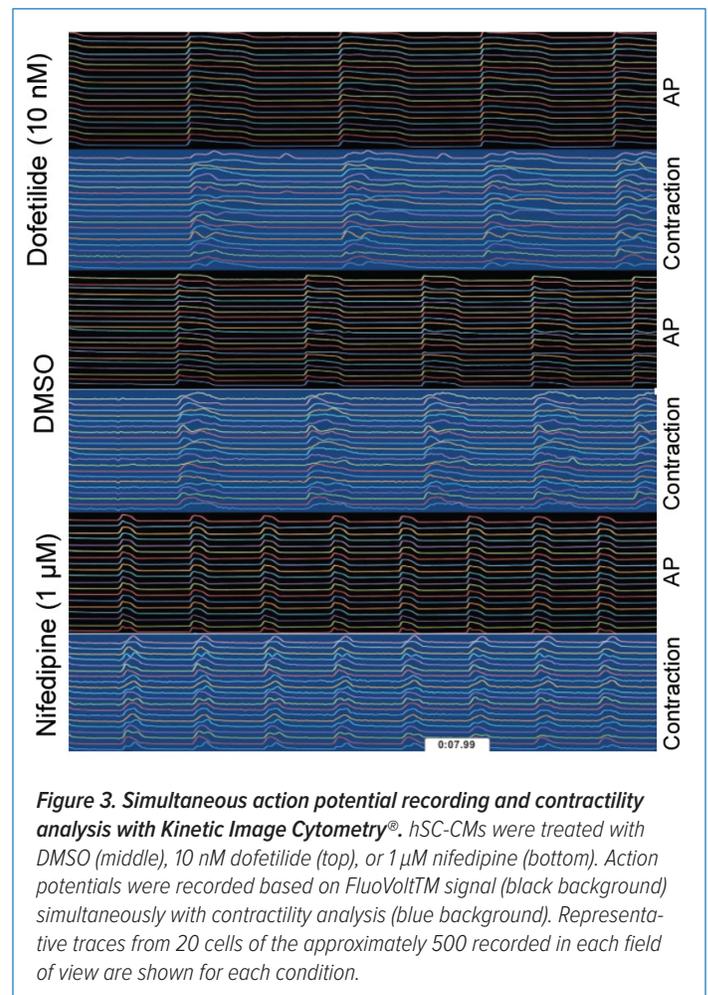

Figure 2. Contractility analysis of hiPSC-CMs labeled with WGA. (A) Vectors represent deformations of the hiPSC-CMs during contraction. (B) Contractility profile, D(t) plotted vs. time. Amplitude (A) and period (T) of contraction are shown.

Images were acquired was with a 20x NA 0.75 objective. The instrument navigated to each well in the dish, captured a single image of the nuclei (Hoechst 33342), and then switched to the green fluorescence channel and acquired images for 10 seconds at 30 frames per second. A single field of view (0.58 mm^2) was imaged per well. Each field of view contained approximately 500 hSC-CMs. Images were analyzed with CyteSeer® (Vala's cell image analysis program), which identifies each nucleus in the field of view and generates a cytoplasmic mask of each cell to enable quantification of changes in FluoVolt™ fluorescence and cell motion.

RESULTS

For initial experiments in which KIC® was used to quantify contractile motion, hiPSC-CMs labeled with WGA were imaged with the KIC® and analyzed with a custom algorithm that tracks displacement of the edges of the cells (Figure 2). The algorithm correlates cell size and position across frames, calculates deformation vectors, and quantifies contractility as the relative change of the measured deformation field. The contractility is used to calculate the amplitude and period of contraction for the cells. This approach successfully identified the negative inotropic effects of tyrosine kinase inhibitors with anti-cancer effects.[11] This contractile motion algorithm is now licensed by Vala Sciences Inc. for use in CyteSeer®, Vala's cell image analysis program.

In a separate approach, Vala researchers developed algorithms for KIC® that simultaneously quantify changes in FluoVolt™ intensity corresponding to cardiac action potentials and spatial displacement of the FluoVolt™ signal at the membrane corresponding to contractile motion of these cells (Figure 3). DMSO-treated hSC-CMs displayed consistent FluoVolt™-detected action potentials of expected frequency and magnitude (Figure 3). Action potential frequency correlated well with contraction frequency in individual cells. The test compounds had the expected effects on hSC-CM action potentials: dofetilide increased their duration and decreased their frequency while nifedipine decreased their duration and increased their frequency. Both compounds had corresponding effects on hSC-CM contractility, and some dofetilide-treated cells aberrantly

contracted between action potentials. These results demonstrate that Vala's IC200 KIC® and CyteSeer® analysis software can detect changes in voltage and contractility simultaneously on a cell-by-cell basis in hSC-CMs with normal and aberrant action potentials.

Figure 3. Simultaneous action potential recording and contractility analysis with Kinetic Image Cytometry®. hSC-CMs were treated with DMSO (middle), 10 nM dofetilide (top), or 1 μM nifedipine (bottom). Action potentials were recorded based on FluoVoltTM signal (black background) simultaneously with contractility analysis (blue background). Representative traces from 20 cells of the approximately 500 recorded in each field of view are shown for each condition.

DISCUSSION

Cardiotoxicity remains a major safety risk for drug candidates,[1,2] but high-throughput methods using hSC-CMs can increase the efficiency and accuracy of preclinical cardiosafety screens. The data presented in this report demonstrate that Vala's instrumentation (the IC200 KIC®) and image analysis software (CyteSeer®) can simultaneously collect cell-by-cell data on action potentials and contractions for hundreds of hSC-CMs and detect the effects of chemical compounds on these parameters. This assay can be expanded to include measurements of intracellular calcium concentration transients in hSC-CMs using the fluorescent calcium sensor Fluo-4.[9,12] CyteSeer® automatically reports many parameters relevant to cardiac physiology, including action potential duration at multiple decay points, upstroke and decay times, contraction amplitude and frequency, and kinetic synchrony across a sample of cardiomyocytes.

CONCLUSIONS

This report lays the groundwork for a single high-throughput assay that captures many of the effects of pharmaceuticals on heart muscle. Vala researchers are developing deep learning methods to identify multi-parametric predictors of drug-induced cardiotoxicity from the multitude of data (including the images themselves and data parameters derived by CyteSeer®) collected by the IC200 KIC®. These predictors can be used for high-throughput screening of drug cardiotoxicity to predict patient susceptibility prior to treatment or to engineer drugs with minimal toxicity across multiple genetic backgrounds.[11] In addition to cardiotoxicity, Vala's IC200 KIC® can also derive data from other excitable cells that are relevant to chronic heart failure, muscular dystrophies, or neurodegeneration. Thus, the IC200 KIC® is a versatile automated microscopy workstation with broad applications across biomedical and cell biology research.

CONTACT US

Please contact Vala Sciences, Inc at www.valasciences.com/contact, info@valasciences.com, or toll-free at (858) 742-8252 for more information about purchasing an instrument, which includes a copy of CyteSeer®. We also provide drug discovery or safety screening services using our technologies.

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