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A Non-invasive Platform for Functional Characterization of Stem-Cell-Derived Cardiomyocytes with Applications in Cardiotoxicity Testing

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SUMMARY

We present a non-invasive method to characterize the function of pluripotent stem-cell-derived cardiomyocytes based on video microscopy and image analysis. The platform, called Pulse, generates automated measurements of beating frequency, beat duration, amplitude, and beat-to-beat variation based on motion analysis of phase-contrast images captured at a fast frame rate. Using Pulse, we demonstrate recapitulation of drug effects in stem-cell-derived cardiomyocytes without the use of exogenous labels and show that our platform can be used for high-throughput cardiotoxicity drug screening and studying physiologically relevant phenotypes.

INTRODUCTION

Recent advances in stem cell technologies have enabled routine analysis of patient-derived cardiomyocytes, opening up new opportunities for drug testing and personalized health care. Numerous studies have demonstrated that induced pluripotent stem-cell-derived cardiomyocytes (iPS-CMs) display physiologically relevant characteristics and patient-derived iPS-CMs recapitulate aspects of patient cardiac pathology/phenotype in vitro (Harris et al., 2013; Navarrete et al., 2013; Sun et al., 2012). iPS-CMs can be used for preclinical testing of new drugs that may cause drug-induced arrhythmia or QT prolongation and cardiotoxicity, as well as for post-market safety testing or repurposing of existing Food and Drug Administration-approved drugs (Guo et al., 2011; Liang et al., 2013; Sirenko et al., 2013; Himmel, 2013). Improved cell-culturing technologies now allow for the production of well-characterized cardiomyocytes at scale, hence providing a reliable source for routine screening applications. Therefore, accurate and reliable characterization of these cells, and their response to different chemical compounds plays a critical role in their successful utilization in drug development and safety testing.

An ideal platform for characterizing iPS-CMs would ensure reproducibility, require small samples, provide a reliable and comprehensive quantitative profile of cell function, and be cost effective when run at large scales. Label-free video microscopy has already been recognized as a well-suited platform (Makino et al., 1999; Hossain et al., 2010). For example, Ting et al. (2014) created a video-management platform that determines whether a specific region is beating; it segments and counts the beating pattern/signal of differentiated cardiomyocytes with a user-specified threshold on the average change in signal intensity. Also, Ahola et al. (2014) captured the beating activity of single cardiomyocytes by analyzing the motion vector field of individual cells manually segmented by the user. Similarly, researchers estimated beating profiles of cardiomyocytes with a block-matching optical flow approach (Huebsch et al., 2014). While this approach yields vector fields of cellular motion for beating monolayer and single-cell iPSC-CMs, it is computationally expensive and may require manual tuning of the expected motion parameters and signal thresholds for each video. These efforts show the promise of video microscopy and analysis; however, we need an integrated and fully automated solution to characterize iPS-CMs at larger scales. This solution must avoid manually tuning software parameters for each video and also handle a broad range of cell-culture conditions, such as varied cell densities and drug treatments. Finally, to facilitate real-time monitoring at relatively low cost, the algorithms used to identify motion must be rapid and suitable for computational implementation without the need for parallel computing.

In current practice, patch-clamp assays are the standard reference for high-precision electrical measurements of iPS-CMs (Peng et al., 2010). However, patch-clamp analysis requires manual operation by a trained electrophysiologist. Such assays are inherently low-throughput and will not scale to meet the demands of large-scale drug testing. iPS-CMs can also be characterized using electrical potentials captured by a micro-electrode array (MEA) (Harris et al., 2013). With an MEA system, the local potential in a region consisting of electrically active cells is measured as a function of time in order to generate a beating signal that

contains information such as frequency, irregularity, and QT interval. Such systems typically require high cell density in specialized plates and rely on direct contact between cells and electrodes. Other methods, such as fluorescence imaging of the calcium signals (Paredes et al., 2008), can be useful, but are prone to phototoxicity as well as potential interactions between calcium indicators and the chemical compounds being studied (Muschol et al., 1999).

In this paper, we present an all-in-one platform, Pulse, which uses video microscopy and image-analysis algorithms (Maddah and Loewke, 2014) to automatically capture and quantify the beating patterns of cardiomyocytes. Our technique generates a beating signal that corresponds to the biomechanical contraction and relaxation of iPS-CMs, based on motion analysis of phase-contrast images captured at up to 50 frames per second. From the beating signal, various quantitative measurements such as beating frequency, irregularity, and duration of a single contraction are calculated. We designed a set of experiments to validate and test the Pulse platform, performed successfully across 800 different videos, and used a diverse set of compounds to investigate the extent to which Pulse can capture dose-dependent responses of different drugs. Pulse is a fully automated biomechanical contractile analyzer designed to be compatible with common cell-culture practices (using standard multi-well plates) and completely non-invasive to cells, making it ideal for large-scale cardiovascular drug development and cardiotoxicity testing. It is worth mentioning, however, that Pulse is not meant to replace electrophysiology or other methods such as MEA or Ca^{2+} imaging altogether, but to supplement them. We envision applications of Pulse as a primary screening tool that can be used to efficiently and cost-effectively scan large numbers of compounds for cardiotoxicity. Such studies could then be followed up in more detail with fewer and more-targeted patch-clamp assays.

RESULTS

Video Motion Analysis

The Pulse video analysis software extracts and quantifies beating signals from the video of cardiomyocytes. Our method enables automated extraction of quantitative parameters that are of interest in clinical studies from cultures with different cell densities and with either regular or irregular beating patterns. Specifically, it captures and quantifies the biomechanical beating of cardiomyocytes by performing motion analysis on the image sequence to capture changes in the image intensity due to cardiomyocyte contraction and relaxation. The design of our algorithm is guided by the fact that it should be possible to work on different tissue types without the need for any parameter tuning. This is why we avoid the use of specific cell segmentation algorithms and apply a more data-driven approach. Figures 1A–1D diagrams the steps of the Pulse algorithm, which includes (1) block-wise segmentation of the image sequence, (2) extraction of the beating signal for each block, (3) quantification of the beating signals, (4) outlier rejection, and (5) clustering of the beating signals into a set of unique signals, each representing a region of the culture where cardiomyocytes beat in synchrony.

Extraction of Beating Signals

The algorithm first segments each image in the video sequence into 30 non-overlapping blocks. Background blocks, i.e., those that do not contain any cells, are removed from further processing. Then a beating signal is extracted for each block as follows. For every image and each candidate beating block, a one-dimensional vector of pixel intensities is constructed. Starting from the second image in the sequence, the correlation coefficient of intensity vectors of the image and its preceding image in the sequence is computed. Figure 1E shows an example of a beating signal extracted for one of the blocks. This *relative correlation* signal typically exhibits three states: a resting state, where the correlation between successive images is high, a contraction state, and a relaxation state. Although the beating pattern and frequency can be measured from this signal, automatic identification of beating intervals is challenging due to the presence of double peaks and the lack of prior knowledge on their relative magnitude or distances. To obtain a single-peak signal, the algorithm first estimates a reference image by taking the median of resting-state images. An *absolute correlation* signal is then generated by computing the correlation coefficient of the intensity vector of the reference image with those of all images in the sequence. Figure 1F shows an example of the resulting signal.

Quantification of the Beating Signal

To capture irregularity and dynamics of beating profile over time, we perform the analysis in the time domain as opposed to frequency domain. The algorithm first identifies the peaks of the absolute correlation signal. A vector of estimated beating intervals is constructed by calculating the duration between successive peaks. We define the effective beating rate based on the median of the vector and define the irregularity of beating pattern based on the interquartile range (IQR) of the vector. We define the beat duration at 75% (meaning the duration is measured at 25% of the peak magnitude in the absolute correlation signal) and take the average duration over all beats. Finally, peak height, which is a unit-less quantity, is a measure of contractility; stronger contraction results in larger values for the peak height. Figure 1F schematically shows how these measurements are performed on the beating signal.