Automatic Single Cell Calcium Transient Analysis for Cardiotoxicity Screening and Pharmacological Testing

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ABSTRACT

High throughput (HT) screening for cardiotoxic drugs and cardiotoxicity using physiological relevant cells is a bottleneck of the modern drug discovery process. Current HT electrophysiological recordings are based on engineered tumor cell lines, underestimating the complexity of cardiomyocyte excitability and physiology. More physiological data are reserved for late-stage development, contributing to large drug failure rates and a cost of over US$ 23.5 billion annually. To obtain high-throughput physiological measurements, we have developed a kinetic Image Cytometer (KIC) for dynamic imaging and automated cell-by-cell analysis of intracellular probes and report its use measuring Ca2+-intra cellular dynamics. The instrument electrically stimulates and acquires intracellular Ca2+-fluorescence from hundreds of cardiomyocytes per well in 96-well plates. The associated software packages the images and measures fluorescence dynamics on individual cells, determining Ca2+ kinetic parameters for nuclear and cytoplasmic compartments and performing statistical analyses on the entire cell population or gated subsets. Experiments performed on human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes using Ca2+ channel blockers (TTX and hERG), hERG blockers (flecainide, cisapride and mepicortil), and calcium channel blockers (nimodipine, diltiazem and ryanodine) validated discrimination of pharmacological effects through HT dynamic imaging analysis. Post-fractionation also enables high-throughput histological correlation of expression markers and individual cell kinetics. This is the first report of a platform that integrates high-throughput and physiological analyses of cardiomyocytes for basic research, as well as drug screening and cardiotoxicity risk assessment.

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REFERENCES: