Novel High Content Stem Cell-Based Assays for Screening

Oksana Sirenko, Pierre Turpin, Jayne Hesley, H. Roger Tang, and Evan F. Cromwell*
Molecular Devices, Inc. Sunnyvale, CA

Introduction

The biopharma industry is continuing to adapt more cell-based assays for primary and secondary screening because of higher biological relevance and increased value of information. As part of this evolution, there is a desire to move from immortalized, stable cell lines to primary cells and stem cells. Stem cells offer the advantages of providing better clinical relevance of information compared to cell lines, being available in larger quantities, and having higher assay reproducibility than primary cells. Accordingly, there is a great interest in automated stem cell assays to use as screening tools in early drug development, and to evaluate potential toxic effects of new compounds.

The workflows for assays involving stem cells are similar to other cell-based assays, but there are critical differences. Differentiation is often involved in stem cell assays because of the need to start with undifferentiated cells, or because the assay is studying the differentiation process. Monitoring the type and number of cells is often important when more than one lineage is present, or when both mature and undifferentiated cells are present. Accordingly, the data analysis and visualization requirements become more complex as multiple readouts from multiple cell types are required.

We have developed high content imaging methods based upon automated image acquisition and multiplexed analysis to perform assays on three types of stem cells: hematopoietic, neuronal, and induced pluripotent stem cell (iPS) derived cardiomyocytes. These methods in combination with optimized cell culture protocols will allow screening and validation of drug candidates in three key areas of drug development: neurogenosis, hematopoiesis, or cardioprotection.

Instrumentation

Fluorescence & Label-Free Imaging by IsoCyte Cytometer

Fast and simple analysis of expression levels of markers on single cells.

High Content Imaging of Neuronal Stem Cells and Cardiomyocytes by SnappExpress® Micro System and MetaXpress® Software

- Images acquired with ImageXpress Micro System cell reader
- High content analysis software and image analysis tools
- 488 Anti-β-Tubulin III: 488 nm Exc., 520 nm Em
- Hoechst dye label for nuclei: 455 nm Es, 455 nm Em
- Images were acquired using standard algorithms from MetaXpress Software
- Neurite Outgrowth – identify number of Neurons & Astrocytes
- Cardiomyocytes – identify number of myocytes, neuron length, branching, etc.

High Throughput SpectraMax® Paradigm Fluorescence Plate Reader

- User configurable cartridges for flexible operation
- user-identifiable LED excitation with dual PMT detector
- Calibrant: 485 nm Exc., 520 nm Em
- Ch1: 550–580 nm (hydrous nitrite) or 590 nm (non-hydrous nitrite)
- Ch2: 500–540 nm (Hydrosol G stain)
- Images were acquired using standard algorithms from Megaview Software
- Neuron counting – identify number of neurons & astrocytes
- Neurite Outgrowth – identify number of myocytes

Stem Cell Hierarchy & Workflow

Expansion

Differentiation

• Aggregate formation

• Self-Renewal

• Myeloid lineage

• Neurogenetic

• Cardiomyocytes

Species

Neuronal Progenitors into Neurons & Astrocytes

Neuronal progenitors (Lina) were expanded in media with EGF and JHDF for 1-2 days and then grown in presence of differentiating media for 12-14 days. The NPCs were cultured into neurospheres with or without EPO to study the effect of EPO on differentiation. Neurons and astrocytes were visualized using ImagesXpress Micro System: 20 images per well were acquired. Representative images from the two differentiation conditions are shown in Figure 2.

The images were analyzed using the Cell Scoring (CS) module and the Neurite Outgrowth (NO) module. Results for two outputs from these modules are shown to the left: 1) Total number of neurons (CD1), CD34+ cells and total neurite outgrowth (NG2). A clear increase in both parameters was observed for cells cultured with EPO.

Differentiation

Hematopoietic progenitors into Myeloids & Erythrocytes

Human BM CD34+ cells were cultured in 96 well format with different hematopoietic cytokines for 5-7 days. The IsoCyte Cytometer was used to scan and analyze the different cell types based on fluorescently labeled markers: Cell population results were further analyzed using FCS Express® 4 Software (De Novo Software) to quantify percentage of each cell type. Results for five cytokine combinations that promote self-renewal and differentiation are shown below in Figure 1.

Stem Cell Assay: Lineage Specific Hematopoietic SC Toxicity

The use of IsoCyte cytometer and FCS Express software can provide information on lineage specific effects of cytotoxic drugs on hematopoietic stem cell differentiation and cell health. Cell-by-cell results are plotted by marker intensities and then populations are analyzed by quadrant gating. Results for differences between three anti-cancer drugs on myeloid and erythroid lineages are shown in Fig 4.

Summary

• The stem cell workflow demands a variety of complex assay types that monitor all phases from initial growth and expansion, to differentiation, to quality control, and finally predictive assays for therapeutic research and drug discovery.
• Molecular Devices offers a suite of hardware and software solutions that enable automated analysis of stem cells for many of the steps in the workflow.
• We are continuing to develop new techniques to help further condense and automate the workflow for stem cell assays.

Differenciation: Cardiomyocytes into Cardiac Cells

IPS progenitors (Celprogen) were expanded in culture with expansion media for 3-7 days. Seven different markers were measured to determine extent of differentiation and maturity of cardiomyocytes. Representative images of Oct4 expression in control, EPO treatment, and a combination, a cardiomyocyte marker, are shown in Figure 5. The markers were identified using mouse or rabbit primary antibodies (Novus) with anti-mouse AP53 or anti-rabbit AP548 (Invitrogen) labeled secondary antibodies. Nuclei were identified with Hoechst dye.

High Throughput Cardiotoxicity

A number of cardiotoxic drugs can effect general cell viability, integrity of mitochondria, and metabolic oxidative activity. These parameters were evaluated by SpectraMax Paradigm plate reader in high throughput compatible assays. In one assay, cells were stained with Calcein AM and Mitotracker in multiplexed assay after 72h incubation with cardiotoxic drugs and viability was determined by total fluorescence intensity. In a second, metabolic oxidative activity was determined by ATP detection assay (ViaLight, Lonza). Results are shown in Figure 6. The cell viability and Mitotracker assays showed similar IC50 values while those for ATP were higher.