APPLICATION NOTE

Multiplexed high content hepatotoxicity assays using iPSC-derived hepatocytes

Introduction

Drug-induced hepatotoxicity is an important cause for liver injury and acute liver failure. Thus highly predictive assays for safety and efficacy testing are crucial for improving drug development and reducing drug attrition. Human induced pluripotent stem cell (iPSC)-derived hepatocytes, which exhibit typical characteristics and metabolism of mature cells, are ideal for use in high-content screening in early drug development.

Although protocols for performing standard assays on high content screening systems are well established, image analyses required to evaluate various forms of toxicity can be complex and custom-driven. In this note, we demonstrate the development of multi-parametric hepatotoxicity assays utilizing the ImageXpress® Micro System. Each well or cell yields multiple compound-induced cellular responses, which are then analyzed using custom modules from MetaXpress® Software.

Getting more information from a standard viability assay

Viability dyes such as Calcein AM can be used to address gross compound toxicity in live cells. iCell Hepatocytes (Cellular Dynamics International) were first treated with various compounds for 72-hours then stained. Images of live cells were acquired using a 10X objective on the ImageXpress Micro System, then total nuclei count (Hoechst counterstain) and cytoplasm area of living cells (Calcein AM) were analyzed using the Multi-Wavelength Cell Scoring Application Module of MetaXpress Software (Figure 1).

Benefits

- **Screen for hepatotoxicity in 96 or 384-well plate format easily and rapidly**
- **Measure multiple hepatotoxicity effects all in one assay**
- **Tailor analysis to report relevant output using custom modules**

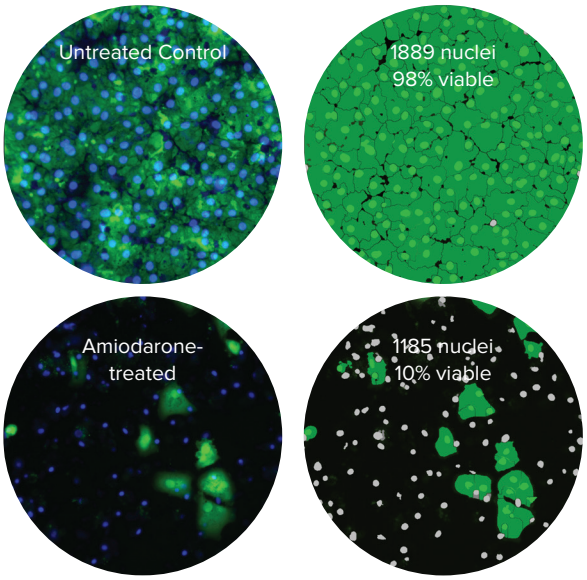


Figure 1. A viability dye yields more accurate toxicity information than nuclear count. Live cells stained with Calcein AM are identified using Multi-Wavelength Cell Scoring Module. The masks on the right show nuclei belonging to live cells in bright green and nuclei in dead cells in grey. Although a similar number of nuclei are counted in control vs. treated wells, it is clear from the percent viable cells that Amiodarone is highly toxic. Additional measurements from the cytoplasm of the live cells (green mask), such as cell area and average intensity can yield insight into the toxic effect mechanism.

Single-dye mitochondrial toxicity assay

Mitochondrial depolarization is an early signal of hypoxic damage or oxidative stress. Mitochondria membrane potential can be assessed by JC-10 dye. In intact mitochondria, orange J-aggregates are visible but, upon membrane depolarization, the dye leaks into the cytoplasm and fluoresces in the fluorescein wavelength. Hepatocytes were treated for 60 minutes with compounds and stained with JC-10 before imaging on the ImageXpress Micro System. A custom module was used to quantitate both the healthy mitochondria which retain the stain and the amount of dye that had leaked into the cytoplasm. The ratio of the two intensities produces a more robust analysis than either measurement alone (Figure 2).

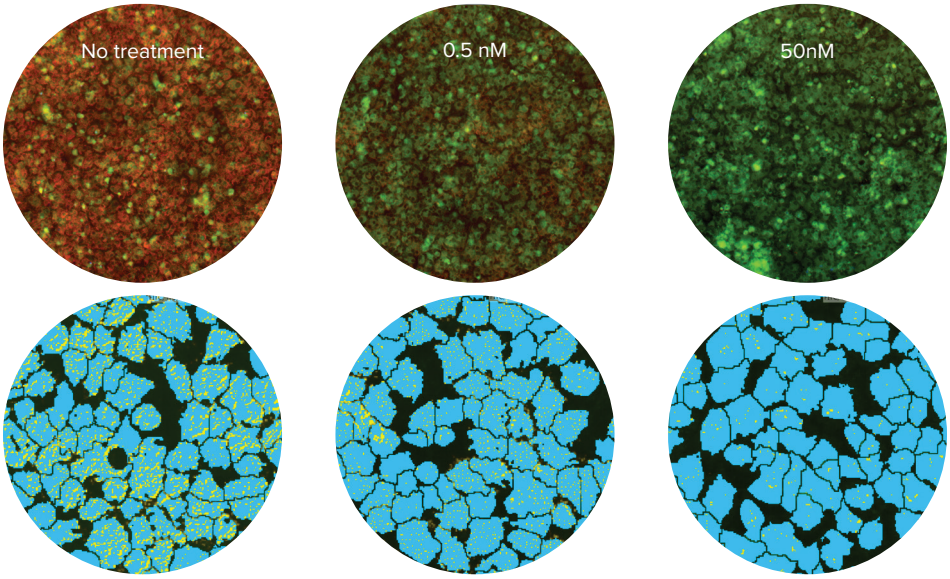


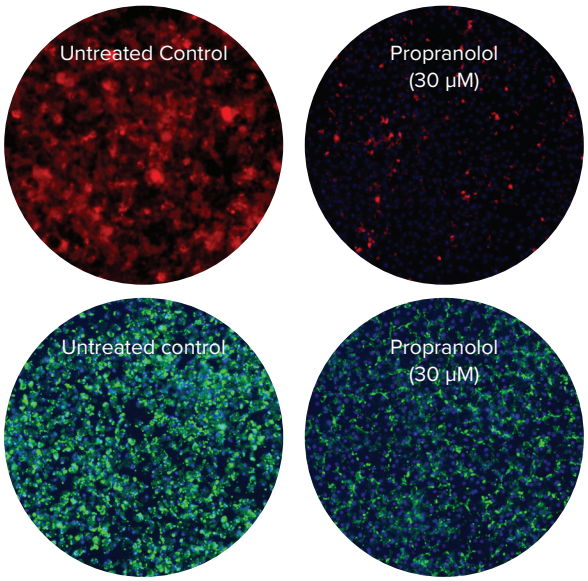
Figure 2. Valinomycin disrupts mitochondria membrane potential. Hepatocytes treated with Valinomycin for 60 minutes. Live cells were stained with JC-10 and imaged with a 10X objective. **Top:** Overlay of green cytoplasm and red mitochondria. **Bottom:** Resulting mask (zoomed) after analysis with a custom module shows mitochondria identified (yellow) in a dose response to the compound.

Phospholipidosis and steatosis are common signs of hepatotoxicity

Some drugs may cause phospholipidosis and steatosis, which are lipid metabolism disorders characterized by excess accumulation of phospholipids and neutral lipids in tissues. The amount and distribution of both phospholipids and neutral lipids in liver cells can be detected using imaging methods and quantitated on a per cell basis. Figure 3 depicts an experiment where hepatocytes were plated in 384-well plates and incubated for four days before treatment with selected compounds for 24 hours.

More information from your toxicity assays

The Custom Module Editor of MetaXpress Software allows scientists to perform multiplexed assays by specifying custom parameters of interest. Custom modules have been demonstrated that quantitate cell size or shape, total number of viable cells, and apoptosis (Figure 4). These modules can be applied to images and run in MetaXpress® PowerCore™ High Content Distributed Image Analysis Software in large batches for high throughput applications.



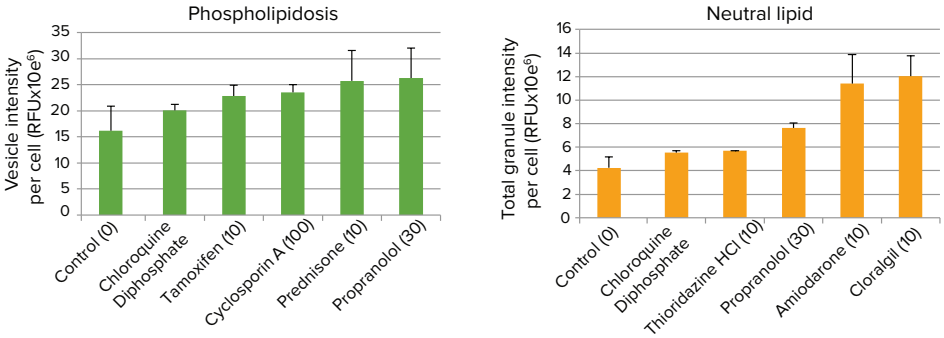


Figure 3. Phospholipidosis and steatosis after compound treatment. Phospholipid **(top)** and neutral lipid **(bottom)** staining in hepatocytes imaged with a 10X objective. Cells positive for lipid staining **(right)** give insight into the specific mechanism of compound toxicity.

A complete solution to multi-parametric hepatotoxicity screening

Multi-parametric image analysis greatly increases assay sensitivity and provides valuable information about mechanisms of compound toxicity. With the Custom Module Editor in MetaXpress Software, scientists have the freedom to design tailored image analysis modules and rapidly analyze toxicity assays to report relevant output only. Molecular Devices® offers flexible high content screening solutions for assessing hepatotoxicity that go far beyond simple live/dead assays.

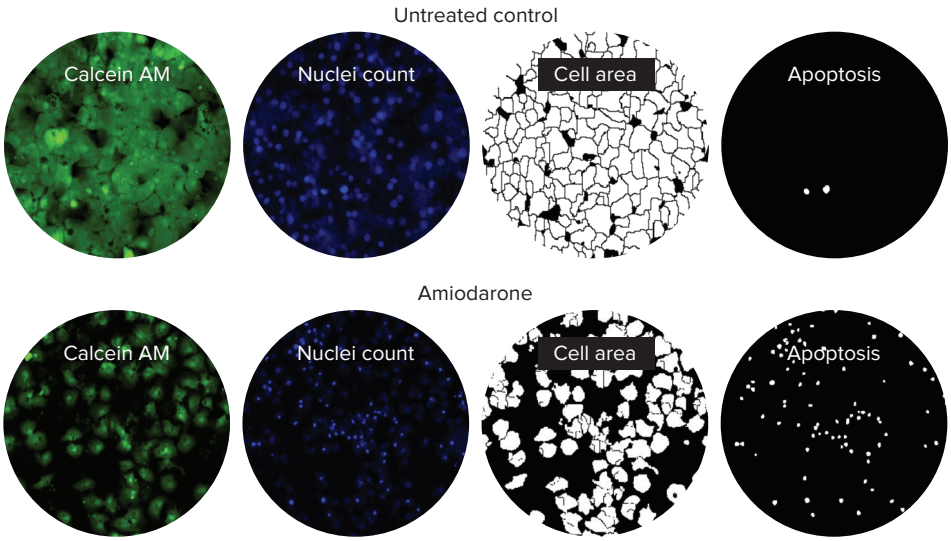


Figure 4. Multi-parametric hepatotoxicity evaluation. This example of a custom module reports measurement of cell area and incidence of apoptosis as well as number of live cells remaining in hepatocytes after compound treatment.

Contact Us

Phone: +1-800-635-5577
Web: www.moleculardevices.com
Email: info@moldev.com
Check our website for a current listing of worldwide distributors.

8/15 0120-1818B
Printed in USA

MOLECULAR DEVICES