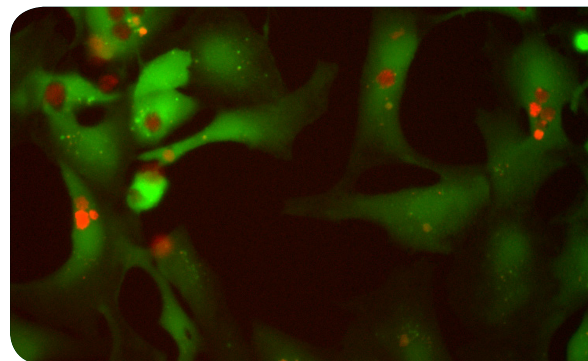
Molecular Devices

Assaying cardiotoxicity with ImageXpress High Content Screening Systems



The discovery and development of novel drugs is a difficult and expensive process due to lack of tools and techniques to accurately reproduce the human physiological response in the laboratory. Off-target cardiotoxicity remains a significant cause of pre- and post approval safety-based drug attrition because of the disconnect between the behavior of cultured immortalized cells and *in vivo* animal models to accurately simulate human cardiac disease states. With whole well imaging and on-the-fly analysis of the ImageXpress® Velos™ Laser Scanning Cytometer or the sub-cellular resolution of the ImageXpress® Micro Widefield High Content System, it is possible to assess the cytotoxic effect of compounds on pertinent human cardiac cells derived from induced pluripotent stem cells.

Toxicity assays

Live/dead assay (gross cytotoxicity)

Membrane permeable Calcein AM is cleaved by esterases in live cells to produce green fluorescence in the cytoplasm. In dead cells, the cell membrane is compromised allowing the dye, ethidium homodimer, to enter and stain the nucleus as seen in Figure 1.

Cardiomyocytes derived from human induced pluripotent stem cells (iCell Cardiomyocytes from Cellular Dynamics Intl.) were incubated with varying concentrations of drugs which were analyzed for cardiotoxicity using the ImageXpress Velos Cytometer in Figure 2 with the data plotted normalized to control in Figure 3. Valinomycin (columns 9 and 10) was highly cardiotoxic even at the lowest levels tested.

JC-10 (cytotoxicity via mitochondria membrane depolarization)

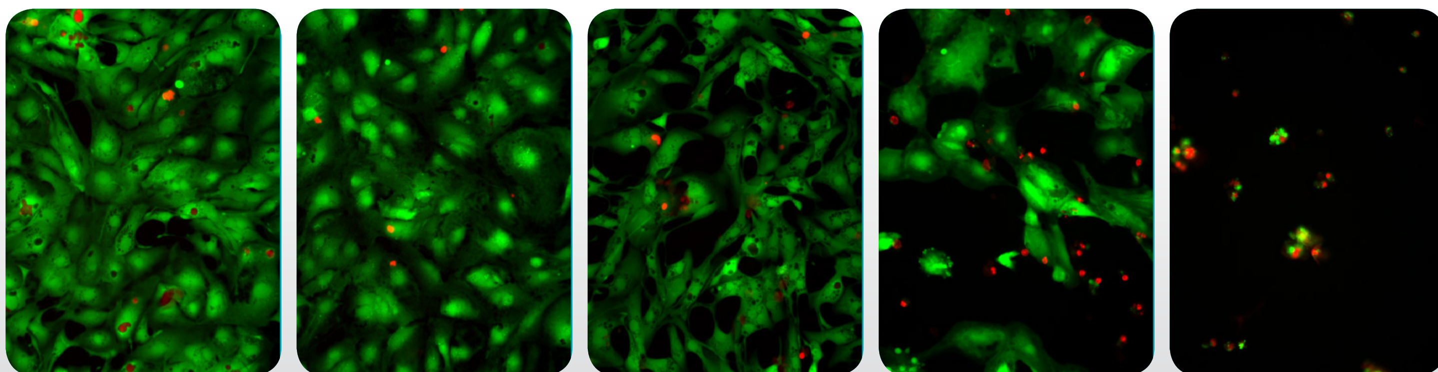
JC-10 aggregates in the mitochondria of healthy cells producing an orange/red emission at 570 nm. Some compounds cause immediate toxic effects by disrupting the membrane potential of the mitochondria. If the mitochondrial membrane is compromised, the JC-10 monomers will be dispersed in the cytoplasm where they emit at 530 nm. Quantitative detection of the mitochondrial membrane change is possible by monitoring the fluorescence intensity ratio at 570 nm/530 nm or simply by quantitating the amount of mitochondria detected per cell.

Cardiotoxicity (Figure 4) was quantitated following just 90 minutes incubation of cardiomyocytes with increasing concentrations of Antimycin A using the ImageXpress Micro System with environmental control (temperature and CO_2). Cells are assayed while alive and acquisition can be scheduled at different time points for evaluation of kinetic effects.

Benefits

- Live-cell assays
- Whole well results
- Automatic image analysis
- Review images later

Figure 1. Cytotoxicity assay



Cardiomyocytes exposed to increasing levels of a toxic compound (left to right). Live cells exhibit green fluorescence and an increasing incidence of red stained dead cells is evident at higher doses. Images of cardiomyocytes were acquired at 20X magnification using ImageXpress Micro Widefield High Content System.

Results

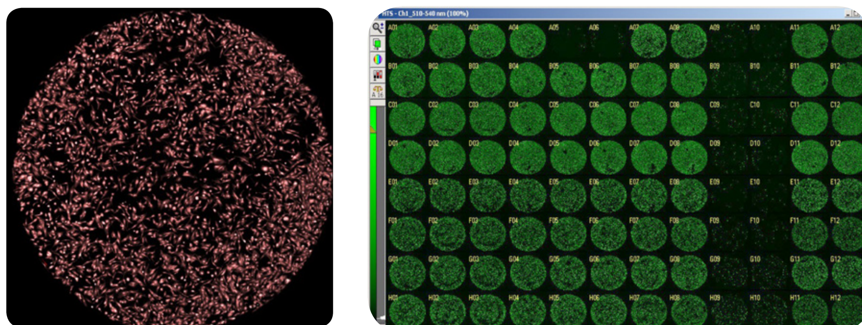
ImageXpress Micro System and ImageXpress Velos Cytometer are powerful systems for analysis of *in vivo* drug cardiotoxicity using cardiomyocytes derived from human induced pluripotent stem cells in both the whole cell cytotoxicity and mitochondria membrane depolarization assays. They provide pharmaceutical scientists with critical information on compound cytotoxicity early in the drug discovery process to help reduce unforeseen cardiac side effects late in clinical trials.

Conclusion

The ImageXpress Micro Widefield High Content Screening System is a fully integrated hardware and software system for automated acquisition and analysis of images for high-throughput cell-based cytotoxicity testing. When configured with the optional environmental control, it can monitor living cell responses or kinetic reactions in real-time for up to several days. Saved images can be reviewed any time and analyzed with one of the MetaXpress® Software Application Modules for toxicity.

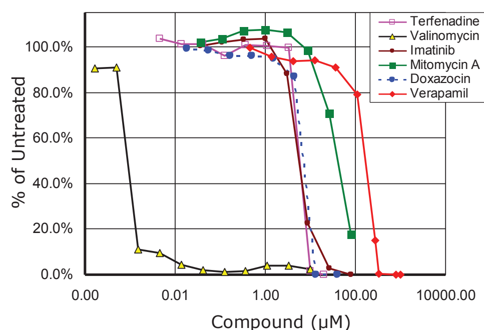
ImageXpress Velos Laser Scanning Cytometer is ideal for performing multiplexed, cell-based assays in a high throughput screening environment. The cytometer can scan each entire well of a whole a plate in as little as three minutes, regardless of cell or well density, making it ideal for simple two color assays such as those shown here. The microplate lid may be left on to maintain sterility during the rapid scan allowing the plate to be returned to the incubator for later rescanning to confirm results or to gather additional time points.

Figure 2. Whole well images of toxicity assay



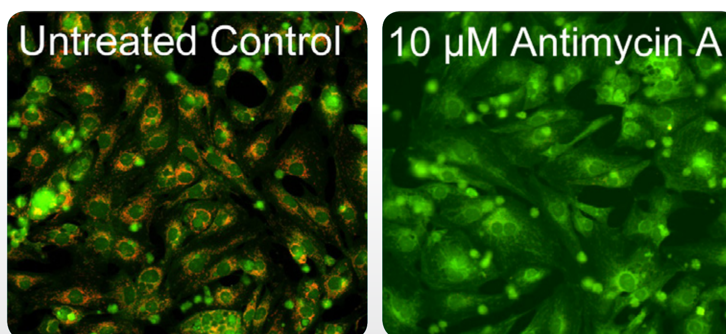
Cardiomyocytes screened against an 8 point dose-response of 5 different compounds tested in duplicate. The compound in columns 9 and 10 was toxic even at the lowest dose tested (row H). Whole well images of cardiomyocytes were acquired using ImageXpress Velos Cytometer.

Figure 3. Toxicity dose response curves



Normalized results of a live/dead assay run on 6 different compounds after 48 hours of drug treatment. Acquisition and image analysis were performed with the ImageXpress Velos Cytometer.

Figure 4. JC-10 mitochondrial membrane potential assay



Increasing concentrations of Antimycin A cause more depolarization of mitochondrial membrane leading to a lower ratio of red stained mitochondria to green fluorescent cytoplasm. Live cell images were acquired at 20X using ImageXpress Micro System with environmental control.

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