Neuronal Cell Imaging & Analysis

High content analysis provides a quantitative method to determine effects of positive and negative factors on neurite outgrowth. An aliquot of Cell Neurons was plated into 96-well plates at 10k/well. The cells were cultured for 3 days and inspected for cell health and maturity of neural networks. Neurons were visualized using ImageXpress Micro XL System and markers for nuclei and β-tubulin III. Image analysis was done using the Neurite Outgrowth module in MetaXpress Software, and data visualization and analysis was done using AcuCyto™ Software. The Neurite Outgrowth module finds nuclei, determines a “positive” neuron cell by presence of both nuclear and β-tubulin III stains, and then characterizes β-tubulin III stained neurons excluding from those cells. Output parameters, in addition to number of neurons, include number of neurites, length of neurites, number of branches, etc. per cell or per field. Statistics on number and phenotype of cells in each well are then calculated. Examples of image analysis results are shown in Figure 2.

Transmitted Light Time Lapse Assay

Live non-stained neurons were imaged using transmitted light. By using transmitted light instead of fluorescent light, data is obtained without risk of disruption to the naturally occurring physiology via addition of foreign molecules. In addition, cell health is not impacted during acquisition as cultures are not subjected to the harsh exposure or free-radical production during fluorescent excitation. Cells were maintained inside the ImageXpress Micro XL instrument with environmental (CO2, humidity, and temperature) control. iCell neurons were plated in the presence of the growth stimulators Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF) and the kinase inhibitor Staurosporine. Effects of the compounds on neurite outgrowth was measured every 20 minutes for 12 hours time period using a 10X objective.

Live Cell Toxicity Assay

End point live cell toxicity assay was done using combination of viability dye Calcein AM and nuclear stain Hoechst. This method is more suitable for screening then β-tubulin III staining as it has a more simple and cost efficient protocol. Neurite networks analyzed using neurite outgrowth module, while content of live and dead neurons determined by nuclear size (Cellometer). We have validated method testing number of known neurotrophic compounds as well as several “safe” compounds. Observed neurototoxic activity of compounds in this model correlated well with in vivo or clinical data showing that the assay can potentially be used for predictive neurotoxicology.

Summary

We have developed high content imaging methods that allow automatic evaluation of neuronal development & toxicity We have demonstrated several automated neurotoxicity assays using iPSC-derived neurons that are suitable for screening environments: - Neuronal network integrity - Mitochondrial integrity and viability markers - Live cell time-lapse assay in transmitted light These assays can be used for: - Testing biologics or chemical compounds on neuronal development - Screening and validation of drug candidates - Evaluating potential neurototoxic or neuroprotection effects of different agents