Assessing Neurite Outgrowth:
Quantification with High Content Analysis

Introduction

Neurons develop inter-neuronal functional networks through neurite outgrowth of axons and dendrites and synapses formation. The regulation of these networks is a major focus of basic neurobiology research on neurodevelopment and neuroplasticity, as well as drug development studies on neurodegenerative diseases and neurotoxicity.

iCell® Neurons are human induced pluripotent stem cell-derived neurons that recapitulate the biochemical, electrophysiological, and pathophysiological characteristics of native human neurons. Due to their human origin, high purity, functional relevance, and ease of use, iCell Neurons represent an optimal in vitro test system for neurobiology interrogations in basic research and in many areas of drug development.

High content analysis (HCA) combines fluorescent microscopy with an automated imaging system to provide qualitative and quantitative analysis of changes in cellular phenotype. With proper handling, iCell Neurons can be thawed and cultured to form neuronal networks amenable to manipulation with target compounds. Together, iCell Neurons and HCA provide an excellent, high-throughput platform for assessing the potential effects of compounds in the modulation of neurite outgrowth and regeneration.

Required Equipment, Consumables, and Software

The following equipment, consumables, and software are required in addition to the materials specified in the iCell Neurons User's Guide.

Item	Vendor	Catalog Number
Equipment		
High Content Imaging System*	Multiple Vendors	
Consumables		
iCell Neurons	Cellular Dynamics International (CDI)	NRC-100-010-001
96 Well Flat Clear Bottom, Black Polystyrene TC-Treated Microplates (96-well Cell Culture Plate)	Corning	3603
Dulbecco's Phosphate Buffered Saline without Ca^{2+} and Mg^{2+} (D-PBS)	Invitrogen	14190
Software		
Data Analysis Software*	Multiple Vendors	

* Several high content imaging systems and data analysis software are available. The cell culturing procedures described here were optimized using the ImageXpress Micro System (Molecular Devices). Data analysis was completed using the MetaXpress Software (Molecular Devices).

Recommended Dyes

The following list of dyes provides the dilution factor to use for staining iCell Neurons.

Item	Vendor	Catalog Number	Dilution Factor
Calcein AM	Invitrogen	C3100MP	1:4000
Ethidium Homodimer-2 (EthD-2) (optional)	Invitrogen	E3599	1:500
Hoechst 33342	Invitrogen	H3570	1:5000

Methods

Culturing iCell Neurons

1. Coat a 96-well cell culture plate with a base layer of 0.01% poly-L-ornithine solution and a top coating of a 3.3 µg/ml laminin solution according to the iCell Neurons User's Guide.
2. Prepare Complete iCell Neurons Maintenance Medium (Complete Maintenance Medium) according to the iCell Neurons User's Guide.
3. Thaw iCell Neurons according to the iCell Neurons User's Guide.
4. Dilute the iCell Neurons cell suspension in Complete Maintenance Medium to a final concentration of 150,000 cells/ml.
5. Aspirate the laminin solution from the 96-well cell culture plate. Immediately dispense the neurons at a density of 45,000 cells/cm^2 (15,000 cells/well).

   Note: *iCell Neurons plating density impacts the neurite outgrowth kinetic.*

Applying Compounds

The following procedure details applying compounds on iCell Neurons cultured in 96-well cell culture plates. Scale volumes appropriately for other vessel formats.

1. Prepare compounds in Complete Maintenance Medium at 10X the final concentration in a 96-well cell culture plate.

   Note: *Final DMSO concentrations above 0.1% should be used with caution. Therefore, if compounds are dissolved in DMSO, the 10X compound solutions should not exceed 1% DMSO.*

2. Transfer 10 µl/well of the 10X compound solutions to the 96-well cell culture plate containing iCell Neurons immediately after plating.
3. Culture iCell Neurons in a cell culture incubator at 37°C, 5% CO_2.
4. Maintain iCell Neurons according to the iCell Neurons User's Guide, partially replacing (50 - 75%) the medium with compounds every 3 days until ready to stain.

Notes

Notes

Staining iCell Neurons

The following procedure details staining iCell Neurons cultured in 96-well cell culture plates for imaging neurite outgrowth with HCA. Scale volumes appropriately for other vessel formats.

1. Aspirate the spent medium. Carefully rinse the neurons once with 100 µl/well of D-PBS at room temperature for 5 minutes.

   Note: *Adding D-PBS too forcefully during this step can easily dislodge neurons from the plate.*

2. Prepare the staining solution by diluting calcein AM solution, Hoechst 33342, and EthD-2 (optional) in D-PBS. Use the dilution factor specified in the above table.

   Note: *Reconstitute 50 µg of calcein AM in 25 µl of DMSO according to the manufacturer's instructions. Use the calcein AM solution on the same day as reconstitution.*

3. Aspirate the D-PBS. Incubate the neurons with 90 µl/well of staining solution in a cell culture incubator at 37°C, 5% CO_2 for 15 minutes.

4. Aspirate the staining solution. Carefully rinse the neurons twice with 120 - 150 µl/well of D-PBS at room temperature for 5 minutes each rinse.

5. Prepare for HCA and analyze within 30 minutes. See the guide for the high content imaging system for data analysis instructions.

Example Data

The data analysis software for the high content imaging system processes the fluorescent signals emitted from the dyes to define cell bodies and corresponding neurite arborization and analyzes the extension of the neuronal network. Figures 1 and 2 exemplify the expected effect of a growth factor (brain-derived neurotrophic factor, BDNF) on neurite outgrowth of iCell Neurons cultured in the presence of the growth factor for 14 days. The neurite outgrowth was measured by calculating the number of branches per neuronal cell, number of processes per soma, and total length of outgrowth per neuronal cell.

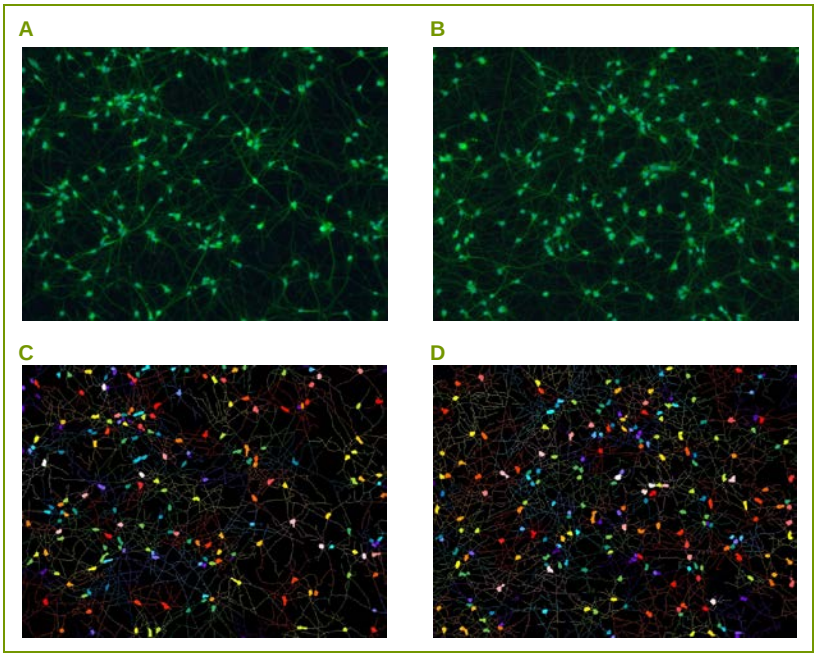

Figure 1: Representative Effect of BDNF on Neurite Outgrowth of iCell Neurons
Panels A and B show iCell Neurons cultured in a 96-well cell culture plate in absence or presence of 50 ng/ml BDNF, respectively. The neurons were stained with calcein AM (green) and Hoechst 33342 (blue). Panels C and D show the segmentation masks generated from calcein AM and Hoechst 33342 fluorescent signals of panels A and B, respectively. Images were acquired using the ImageXpress Micro System, and segmentation masks were generated using the MetaXpress Software.

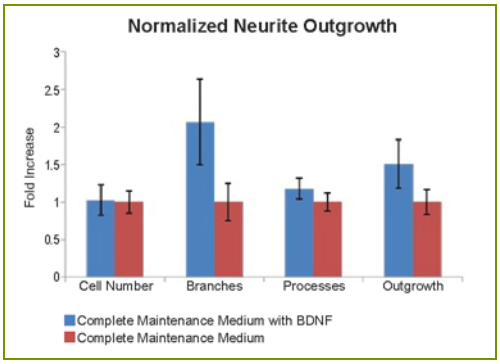

Figure 2: Quantitative Effect of BDNF on Neurite Outgrowth of iCell Neurons
Culturing iCell Neurons in the presence of BDNF for 14 days produced the expected effect of enhancing neurite outgrowth. Data were acquired using the ImageXpress Micro System and quantified using the MetaXpress Software. Data are represented as mean values per number of neuronal cells (mean ± SEM, n = 6 wells).

Notes

Notes

Summary

iCell Neurons provide an in vitro test system that recapitulates native human neuronal cell physiology and function. Neuronal networks of iCell Neurons are established in culture, and functional responses to compounds can be robustly assessed and quantified using HCA. The methods and data presented here highlight the ease of use with which HCA can gather relevant data on neurotoxicity, neuroprotection, or regeneration in living human neurons.

Version: November 2013
AP-NRCGRO131108