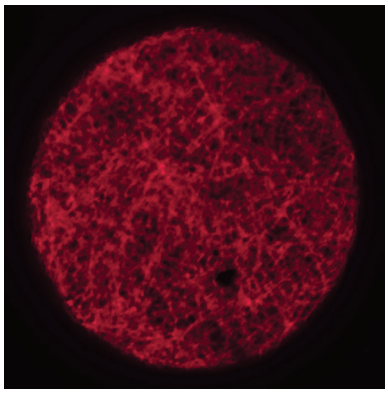

High-content imaging and analysis of 2D and 3D neuronal cultures grown in optimized media

Introduction

The ability to grow neurons in culture, and to monitor and quantify neurite outgrowth, aids in both the understanding of nervous system function and the development of treatments for neurological disorders such as Alzheimer's and Parkinson's diseases [1]. Synaptic growth and neural toxicity can be monitored by growing neurons in culture with the aid of optimized neuronal media and supplements, followed by fluorescent labeling and imaging. Recent developments in 3D culture systems and supports have enhanced the ability to grow neurons in a matrix that more closely resembles the state of nervous system tissue in intact organisms, without the need to use expensive and time-consuming animal models. High-content analysis (HCA) systems can be used to obtain high-resolution images of 2D and 3D neuronal cultures and perform quantitation and complex analysis of key parameters of neurite outgrowth for hundreds of samples in a relatively short period of time [2,3].

The Thermo Scientific™ CellInsight™ CX7 LZR HCA Platform is a 7-laser high-content imaging instrument that reduces scanning times and improves axial resolution in 2D and 3D cultures. These features provide the speed and sensitivity necessary to analyze neuronal culture systems for more physiologically relevant drug discovery assays, and allow tracking and quantification of the dynamic changes in neurites and synapses underlying synaptogenesis or synaptic damage. Here we discuss use of the new-generation Gibco™ B-27™ Plus Neuronal Culture System to generate 2D and 3D neuronal cultures with enhanced growth. These neuronal cultures can then be stained with a variety of fluorescent markers and probes for high-throughput imaging so that neuronal growth, length, and toxicity can be quantified using HCA, allowing data to be obtained from hundreds of neuronal samples over a short period of time.

Materials

- Gibco™ Primary Rat Cortex Neurons (Cat. No. A1084002)
- Gibco™ Primary Rat Hippocampal Neurons (Cat. No. A36513)
- Gibco™ StemPro™ Neural Stem Cells (Cat. No. A15654)
- B-27 Plus Neuronal Culture System (Cat. No. A3653401, contains Gibco™ Neurobasal™ Plus Medium and B-27™ Plus Supplement)
- Gibco™ Neurobasal™ Medium (Cat. No. 21103049)
- Gibco™ B-27™ Supplement (50X) (Cat. No. 17504044)
- Gibco™ CultureOne™ Supplement (100X) (Cat. No. A3320201)
- Gibco™ N-2 Supplement (100X) (Cat. No. 17502048)
- Gibco™ BDNF Recombinant Human Protein (Cat. No. 10908010)
- Gibco™ GDNF Recombinant Human Protein (Cat. No. PHC7044)
- Gibco™ CNTF Recombinant Human Protein (Cat. No. PHC7015)
- Invitrogen™ MAP2 Polyclonal Antibody (Cat. No. PA5-17646)
- Invitrogen™ HuC/HuD Monoclonal Antibody (Cat. No. A21271)
- Invitrogen™ Alexa Fluor™ 488 Goat Anti–Mouse IgG (H+L) Secondary Antibody (Cat. No. A11001)
- Invitrogen™ Alexa Fluor™ 555 Goat Anti–Mouse IgG (H+L) Secondary Antibody (Cat. No. A21424)
- Invitrogen™ Alexa Fluor™ 488 Goat Anti–Rabbit IgG (H+L) Secondary Antibody (Cat. No. A11034)
- Invitrogen™ Alexa Fluor™ 647 Goat Anti–Rabbit IgG (H+L) Secondary Antibody (Cat. No. A21244)
- Thermo Scientific™ Nunclon™ Sphera™ 96-Well Round U-Bottom Plates (Cat. No. 174925)
- Invitrogen™ Tubulin Tracker™ Deep Red (Cat. No. T34077)
- Invitrogen™ Live Cell Imaging Solution (Cat. No. A14291DJ)
- CellInsight CX7 LZR HCA Platform (Cat. No. CX7A1110LZR)

Methods

Growth of neuronal cultures in B-27 and B-27 Plus systems

Cryopreserved rat cortical or hippocampal neurons were cultured in the B-27 system (Neurobasal Medium with B-27 Supplement) or B-27 Plus system (Neurobasal Plus Medium with B-27 Plus Supplement) for 3–4 weeks. The cells were fixed with 4% formaldehyde for 15 min, permeabilized with 0.5% Triton™ X-100 detergent for 10 min, and then immunostained with HuC/HuD and MAP2 primary antibodies followed by Alexa Fluor 555 goat anti-mouse and Alexa Fluor 488 goat anti-rabbit secondary antibodies, respectively. The samples were imaged on a CellInsight CX7 LZR HCA Platform.

Monitoring neuronal toxicity

Cryopreserved rat cortical neurons were grown for 2 days and then treated with cadmium chloride or paclitaxel for 24 hr. The cells were then fixed and stained with HuC/HuD and MAP2 primary antibodies followed by Alexa Fluor 488 goat anti-mouse and Alexa Fluor 647 goat anti-rabbit secondary antibodies, respectively. The samples were imaged on a CellInsight CX7 LZR HCA Platform.

3D neurosphere culture and Tubulin Tracker Deep Red staining

Neural stem cells (NSCs) were grown on Nunclon Sphera U-bottom plates (1.3 x 10^4 cells/well) to form neurospheres. The cells were differentiated in the B-27 system or B-27 Plus system, each with or without CultureOne Supplement. N-2 Supplement, growth factors BDNF, GDNF, and CNTF (all 10 µg/mL), and 10 µM ROCK inhibitor (Y-27632) were also added to the cultures. The neurons were differentiated for 2 weeks and stained with 1 µM Tubulin Tracker Deep Red for 1 hr. The spheroids were then washed with Live Cell Imaging Solution and imaged on the CellInsight CX7 LZR HCA Platform.

High-content imaging and analysis

The neuronal cells were analyzed on a CellInsight CX7 LZR HCA Platform using the neuronal profiling bioapplication at 10x magnification. A total of 200 cells were imaged per well, and neuronal cell bodies and neurite outgrowth were measured using their respective parameters. The nuclei were segmented with DAPI, and neuronal cell bodies were identified with HuC/HuD-positive staining. Neurite length was measured with MAP2 staining. For 3D neurosphere imaging, confocal mode was used, where each image represents a maximum intensity projection (MIP) of thirty Z optical slices of 10 µm each.

Results

2D neuronal cultures immunostained with MAP2 and HuC/HuD antibodies to label dendrites and neuronal cell bodies were imaged on the CellInsight CX7 LZR HCA Platform and analyzed with Thermo Scientific™ HCS Studio™ 2.0 Cell Analysis Software to obtain measurements of neurons per field and neurite length. Both rat cortical and hippocampal neurons had increased growth, as indicated by neurons per field, when grown in the B-27 Plus Neuronal Culture System compared to the original B-27 system (Figure 1).

Neuronal toxicity can also be quantitated by imaging and analysis on the CellInsight CX7 LZR HCA Platform and HCS Studio 2.0 Cell Analysis Software as demonstrated by treating rat cortical neurons with 10 mM cadmium chloride or 10 µM paclitaxel. Both treatments significantly decreased the total neurite length per field and percentage of neurons positive for HuC/HuD, demonstrating the neuronal toxicity of these compounds (Figure 2).

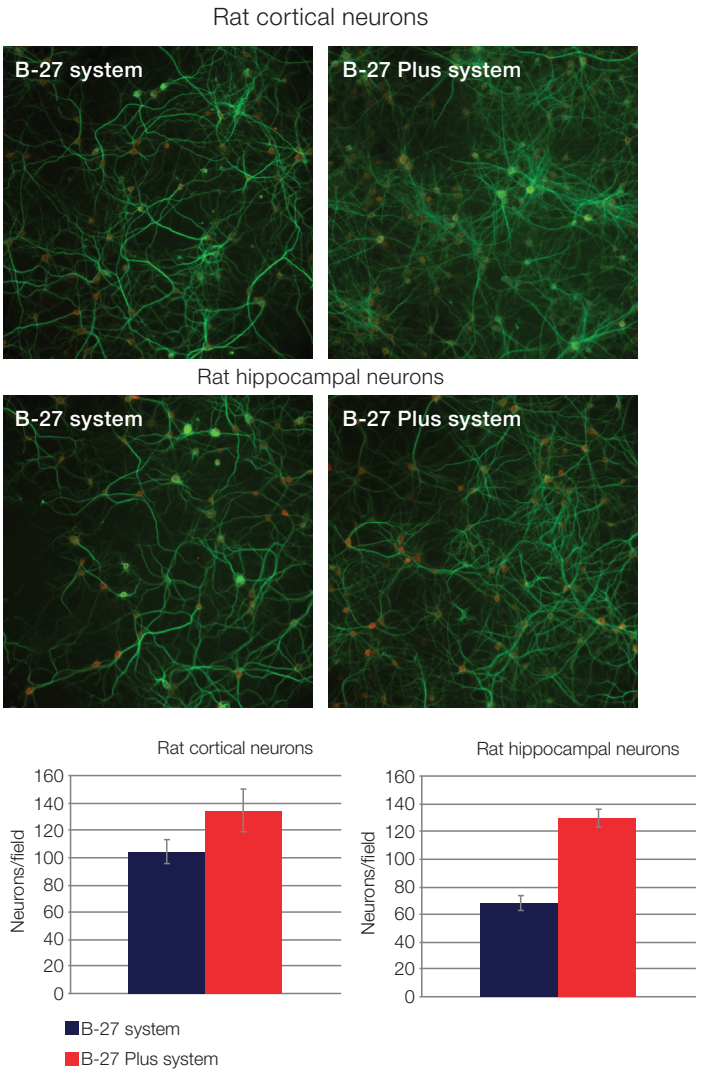

Figure 1. The B-27 Plus Neuronal Culture System enables superior growth of rodent primary cortical and hippocampal neurons, compared to the original B-27 system. Cryopreserved neurons were cultured for 3–4 weeks using the first-generation B-27 system (Neurobasal Medium with B-27 Supplement) or B-27 Plus system (Neurobasal Plus Medium with B-27 Plus Supplement). Neurons were fixed and immunostained for the neuronal dendritic marker MAP2 (green) and the neuronal cell body marker HuC/HuD (red). Fluorescence imaging and quantitation of neurons per field were performed on the CellInsight CX7 LZR HCA Platform. Both cultures have more neurons per field when grown in the B-27 Plus system than in the original B-27 system.

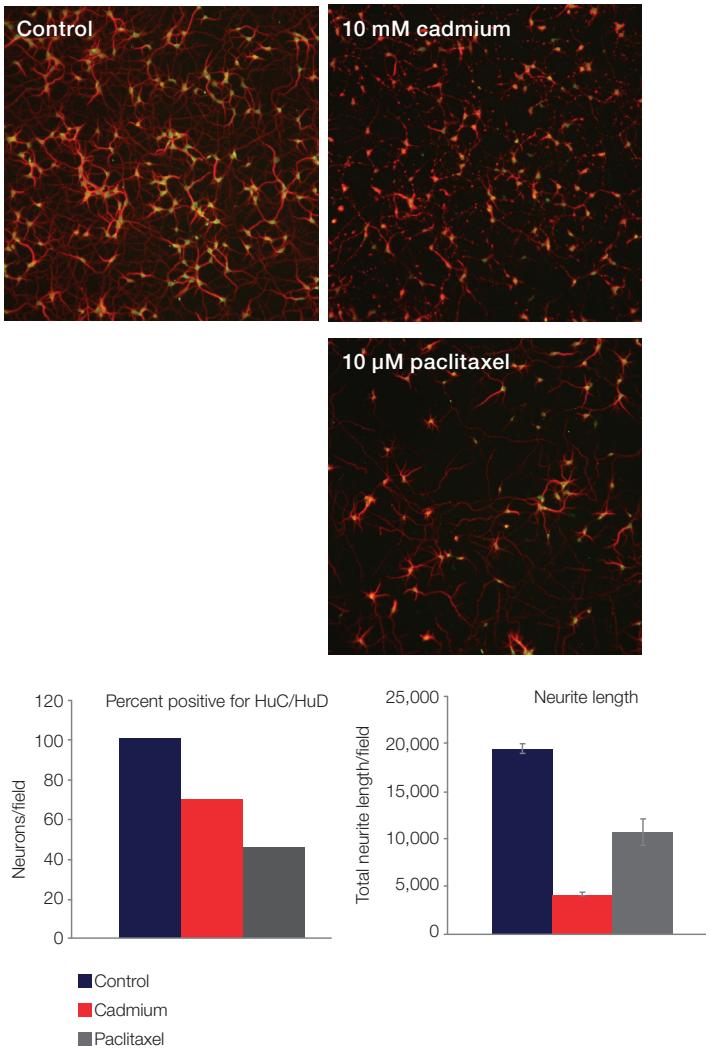

Figure 2. Monitoring neuronal toxicity with the CellInsight CX7 LZR HCA Platform. Rat cortical neurons were cultured for 2 days in a 96-well plate and treated with 10 mM cadmium chloride or 10 µM paclitaxel for 24 hr. Neurons were then fixed and immunostained for the neuronal dendritic marker MAP2 (red) and neuronal cell body marker HuC/HuD (green). Fluorescence imaging and quantitation were performed on the CellInsight CX7 LZR HCA Platform in confocal mode. Segmentation of the neuronal processes allows quantitation of total neurite length per field and percentage of neurons positive for HuC/HuD. Treatment with cadmium or paclitaxel significantly decreased the total neurite length per field and percentage of HuC/HuD-positive neurons, indicating that both treatments were toxic.

Neurons were also grown in 3D culture to form neurospheroids that were stained with Tubulin Tracker Deep Red to label neuronal processes in live cells without the need for fixation or immunostaining. As demonstrated by staining with Tubulin Tracker Deep Red and imaging on the CellInsight CX7 LZR HCA Platform, the 3D neurospheroids showed enhanced growth when grown in the B-27 Plus system with CultureOne Supplement, compared to neurospheroids grown in the original B-27 system, or without the supplement (Figure 3).

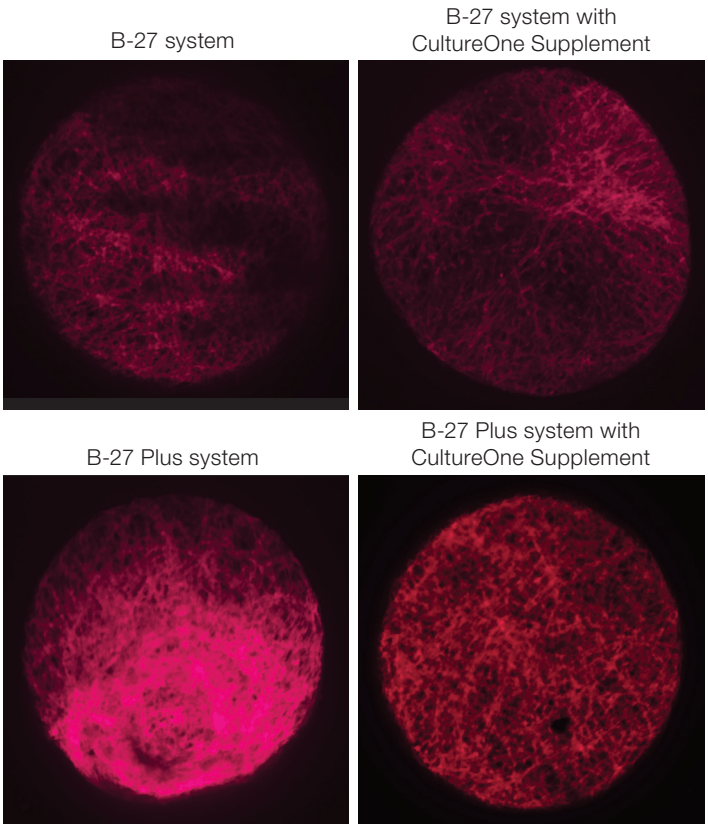

Figure 3. The B-27 Plus system with CultureOne Supplement enhances growth of neurospheroids in 3D culture. Neurospheroids were grown in the B-27 system or B-27 Plus system, each with or without CultureOne Supplement, on Nunclon Sphera U-bottom plates and stained with Tubulin Tracker Deep Red. Imaging of the stained neurospheroids on the CellInsight CX7 LZR HCA Platform shows enhanced neurite growth of the 3D neuronal cultures when grown in the B-27 Plus system with CultureOne Supplement.

Conclusions

High-content imaging and analysis with the CellInsight CX7 LZR HCA Platform and HCS Studio 2.0 Cell Analysis Software enable fast, high-resolution imaging of both 2D and 3D neuronal cell cultures along with automated quantitation and complex analysis of several parameters of neuronal growth and health, including identification of neuronal cell bodies, neurons per field, and neurite length. The B-27 Plus Neuronal Culture System allows superior growth of 2D and 3D neuronal cultures over longer periods of time than the original B-27 culture system. Tubulin Tracker Deep Red, a docetaxel-based fluorescent reagent for live-cell labeling of microtubules, provides neuronal labeling throughout live 3D neurospheroids without the need for fixation or immunostaining. These optimized neuronal culture systems and fluorescent staining reagents combined with high-resolution automated imaging and analysis allow for investigation of neuronal growth and toxicity of hundreds of samples over a relatively short period of time, helping to enable new discoveries in neuronal function and neuropharmacology. These discoveries may potentially be applied to understanding of the nervous system and treatment of neurological disorders.

References

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