INTRODUCTION

The absence of physiologically relevant in vitro models of the nervous system is an important limitation in understanding mechanisms of neurological diseases and drug development. This has generated an increasing interest in using three-dimensional (3D) cultures for assay development applicable for neurodegenerative disease and neurotoxicology screens. The goal of the present study was to develop a model for 3D neurite outgrowth assay using iPSC-derived neurons in the microfluidic, high-throughput OrganoPlate® platform. The OrganoPlate® was developed as an organ-on-a-chip platform allowing the formation of three-dimensional (3D) microfluidic-based, long-term cultures of live cells suitable for screening. Neuronal cultures were treated for five days with several compounds including methyl mercury and other selected chemicals to assess the neuronal viability and complexity of networks, cells were stained using a combination of three dyes: Calcein AM, MitoTracker Orange, and Hoechst dye. The methods used to allow for the measurement of viability, morphology and neuronal health in 3D matrix using automated confocal imaging and analysis. Disruption of neuronal connections were visible in a dose-dependent manner after treatment with neurotoxic compounds. A series of confocal images were automatically acquired at different planes separated by 3-10 µm, covering approximately 150-300 µm in depth. Images were analyzed using our 3D analysis module in ImageXpress analysis software.

RESULTS

Phenotypic Assays in 3D Cultures

High-content imaging and analysis were used for evaluation of treatment effects on neuronal networks. We optimized confocal imaging and analysis protocols for assessing morphology and viability of neurons in this 3D matrix. Images were acquired using ImageXpress Micro Confocal system (Molecular Devices, Sunnyvale, CA), with 10x, 20x or 40x objectives.

A series of images was acquired at different planes along the focal axis (Z-stack) (Figure 1). A stack of 17-30 planes separated by 3-10 µm was acquired, covering approximately 150-300 µm in depth.

3D Visualization and 3D Image Analysis

Molecular Devices software (Molecular Devices) offers a 3D analysis option that allows for combining objects from adjacent Z-planes, as well as 3D visualization of cells and networks. Images were analyzed using a 3D analysis Custom Module using “find fibers” function with which a “fibers” measurement was generated for quantifying neurite outgrowth. Objects are first found in each plane, and then connected in 3D space using the “connect by best match” function. The measurements output from the 3D analysis included the number of neurites (fibers) and processes, cell volumes, total volume of fibers, numbers of processes, nuclei, and branching points.

Comparison between 2D and 3D Models

Disruption of neuronal networks, cell viability, and mitochondrial potential markers were compared between conventional 2D cell cultures and 3D cultures in Organoplates. EC50 values for toxicity effects for retinoic acid and paracetamol were relatively higher for 3D cultures, and lower for rotenein, while for other compounds the values were comparable.

CONCLUSIONS

We presented here the methods that will enable neurodevelopment and neurotoxicity assays that use 3D models.

Confoocal imaging and 3D analysis allows quantitative characterization of complex phenotypic effects

3D neuronal models can be successfully used for toxicity evaluation, disease modelling, and compound screening.