Fully automated development of iPSC-derived 3D neural organoids and functional analysis of calcium oscillation activity

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Introduction

Growing neural 3D organoids from human induced pluripotent stem cells (iPSC) is a rapidly developing technology with great potential for understanding brain development, neuronal diseases, and impact of different genetic backgrounds. However, the formation of neural organoids is a complex and lengthy process, making it less suitable for compound screening. Here we demonstrate an automated protocol for development of neural organoids (scaffold-free) using the CellXpress.ai® Automated Cell Culture System. The device has liquid handling, imager, automated incubator, and process-controlling scheduling software. The device enables automation of most repetitive tasks, including organoid culture with periodic media exchanges, agitation, and organoid development monitoring by imaging. The protocol includes media exchanges and monitoring by imaging. After maturation of organoids, functional characterization of spontaneous neural activity of 3D organoids was measured by recording and analysis of calcium oscillations. Calcium oscillations were recorded on the FLIPR® Penta High-Throughput Cellular Screening System that measured fast kinetic changes in calcium signal. Oscillation patterns were analyzed for multiple parameters including peak count, amplitude, and peak width. Organoids demonstrated spontaneous calcium oscillation activity after day 60 in culture.

Morphological characterization of 3D organoids was done by imaging. Organoids diameters ranged from 1800–2000 µm, and the expression of neural markers including TUJ1 and GAFP were detected with fluorescently labeled antibodies. For pharmacological characterization, several compounds were used to show the appropriate functional responses. AMPA and 4-AP addition resulted in dose-dependent increase of frequency of calcium oscillations, while GABA and caused decrease in oscillation frequencies. Taken together, this biological system of iPSC-derived 3D neural organoids paired with process automation and detailed analysis of calcium oscillations demonstrates a promising tool for compound testing.

Instruments

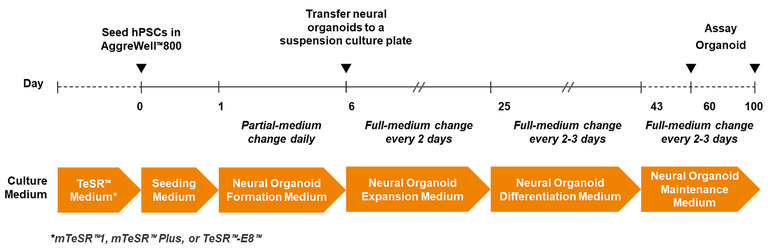
CellXpress.ai Automated Cell Culture System

HCS.ai Automated Imaging System

FLIPR Penta provides fast kinetic read-outs

Methods

Organoid culture: Human iPSCs (ATCC. ACS-1023 A) were maintained in mTeSR Plus, dissociated, and seeded at a density of 3 × 10^6 cells/well in Seeding Medium (Formation Medium supplemented with 10 µM Rho-kinase inhibitor (ROCKi)) in AggreWell800 plates. Dorsal forebrain organoids were generated using the STEMdiff Dorsal Forebrain Organoid Kit (STEMCELL Technologies, Catalog #08620) that includes several media components. Cell aggregates were first fed daily with Formation Medium. After 6 days, organoids were transferred to 6-well plates in Expansion Medium and maintained in the CellXpress.ai system incubator with periodic feeding and imaging. Organoids were fed every 2 days with Expansion Medium until day 25, followed by Differentiation Medium until day 43. From day 43 onward, Maintenance Medium was used every 2–3 days. After 50 days in culture, organoids were maintained in BrainPhys media (STEMCELL Technologies). The flowchart below represents the STEMdiff Dorsal Forebrain Organoid protocol.



**mTeSR™I, mTeSR™Plus, or TeSR™-E8™*

Functional characterization of neural activity by calcium imaging. On the day of assay, spheroids were loaded with CellXpress.ai Automated Cell Culture System. The CellXpress.ai system contains a liquid handling component, an automated incubator, an embedded imager, and automated tools providing transport plates from the incubator to the imager or liquid handler and back. The system can perform the essential tasks for cell culture, including cell plating, media exchanges, passaging, and monitoring by imaging. For organoid protocol we automated liquid exchanges and monitoring.

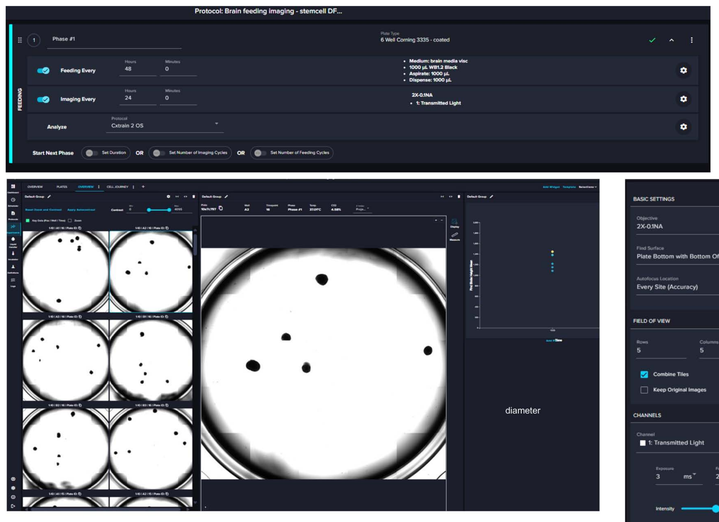


Figure 2. Automated protocol for maintenance and monitoring of brain organoids. Screenshots of the media exchange protocol containing periodic feeding (media exchange), imaging, and image analysis steps. Media exchanges were performed with 1 mL pipette tips, after tilting the plates. Imaging was done with a 2X objective in transmitted light. Image analysis identified organoids and took measurements, including area, diameter, and densities.

Results

Automated development of iPSC-derived 3D dorsal forebrain organoids

Dorsal forebrain organoids were developed from iPSC cells (ATCC) using the STEMdiff Dorsal Forebrain Organoid Kit from STEMCELL Technologies Canada Inc. The process contains several development steps: iPSC maintenance, seeding cells into Aggrewell V bottom plates, formation spheroids (days 0–6), then transferring spheroids into 6-well plates and continuous culture with periodic media exchanges during expansion period (days 6–25), differentiation period (days 26–43), then maintenance period (days 43–60+).

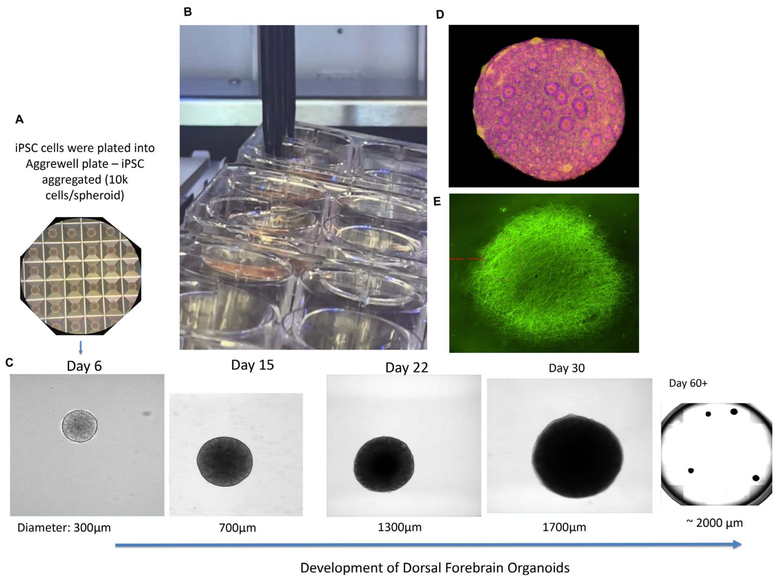


Figure 1. The workflow automation was done using the CellXpress.ai system. The STEMdiff Dorsal Forebrain Organoid Kit (STEMCELL Technologies) was used to generate dorsal forebrain organoids starting from the iPSCs line from ATCC., ACS-1023 A. iPSCs maintained in mTeSR Plus were dissociated and seeded manually into AggreWell800 plates and cultured for 6 days with Formation Medium. B. After 6 days, organoids were transferred to 6-well plates in Expansion Medium and maintained in the CellXpress.ai system incubator with periodic media exchanges with appropriate media and imaging. C. Organoid images were taken at different timepoints. D. Endpoint characterization for morphology was done by imaging after staining with neuronal, astrocyte and projection markers. E. Functional characterization was performed using calcium oscillations recording by imaging or FLIPR assays.

Organoid maintenance and imaging with the CellXpress.ai system

We automated repetitive steps for organoid culture including media exchanges and periodic imaging using the CellXpress.ai Automated Cell Culture System. The CellXpress.ai system contains a liquid handling component, an automated incubator, an embedded imager, and automated tools providing transport plates from the incubator to the imager or liquid handler and back. The system can perform the essential tasks for cell culture, including cell plating, media exchanges, passaging, and monitoring by imaging. For organoid protocol we automated liquid exchanges and monitoring.

Results

Morphological characterization of dorsal forebrain organoids

Functional and morphological characterization was done at different time points of the culture. Staining with SOX2, TuJ1, MAP2, and GAFP antibodies demonstrated the presence of neurons (TuJ1) and astrocytes (GAFP), also the presence of progenitor cells (SOX2 positive), which decreased during the continuous culture.

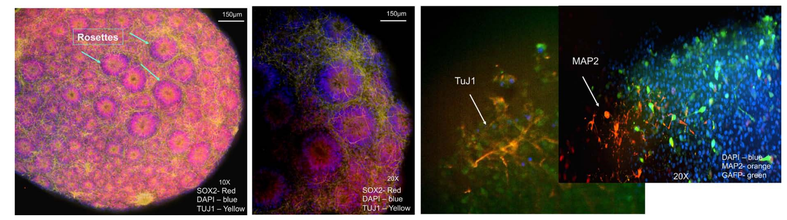


Figure 3. Dorsal forebrain organoids were derived from iPSC cells and then stained. Imaging was performed using the ImageXpress HCS.ai Confocal System at various magnifications. Dorsal forebrain organoids were stained using 4 markers. Undifferentiated organoids (15 days) showed rosettes with predominantly SOX-2 signal. Differentiated organoids, 100 days old, did not show the SOX2 signal. GFAP-positive cells are detected in mature neurons. GFAP-marked astrocytes were absent in the undifferentiated organoid (left) but present in the differentiated organoid (right). TuJ1 (βIII-Tubulin) labeled neuronal cytoskeleton was observed in both organoids. MAP2 marked dendrites were observed in differentiated organoids (right) and SOX2 identified progenitor cells were predominantly expressed in the undifferentiated organoid.

Recording calcium oscillations with the FLIPR system or the ImageXpress Confocal HT.ai system

Functional characterization was done at different time points after 45 days of culture. Sporadic firings were recorded using confocal imaging (see below). After day 70 in culture, organoids were showing more synchronous activity which could be recorded by the FLIPR instrument. Also, we observed expected changes in oscillation activity caused by compounds.

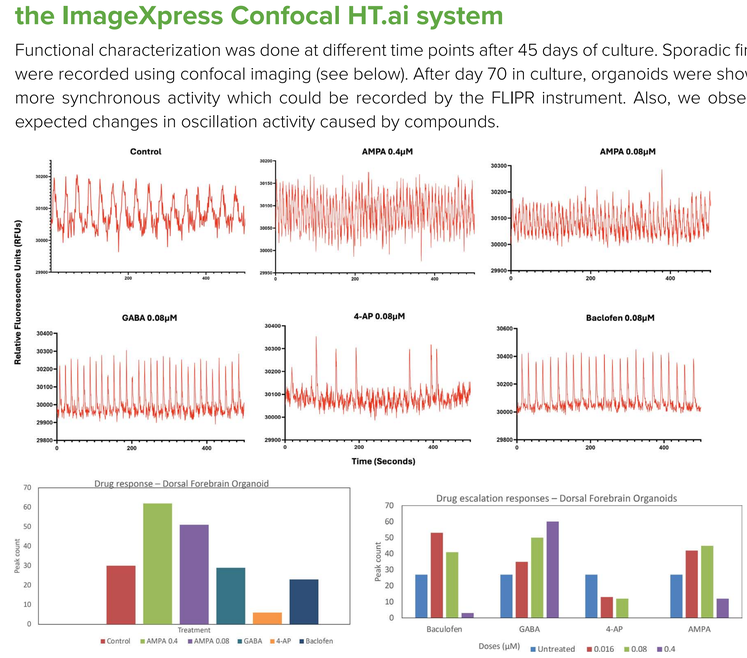


Figure 4. Calcium oscillations were recorded after loading organoids with Calcium 6 dye for 2h. Recordings were taken by the FLIPR Penta instrument. Kinetic readings were taken every 0.4 second. Readings were taken after treatment with 80nM concentrations of indicated compounds for 30 min. Bar graphs above show responses to indicated benchmarking drugs by effects on peak counts.

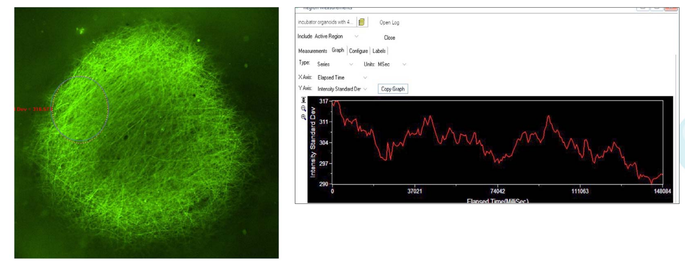


Figure 5. Calcium oscillations were recorded after loading organoids with Calcium 6 dye for 2h. Images acquired with 10x magnification, confocal option, with timelapses every 0.5 seconds. The neurites were stained with Calcium dye (green). The graph shows time-course of standard deviation of the fluorescence taken from the selected region of interest (shown with a circle). This method allows the detection and visualization of asynchronous activity in firing neurons.

Summary

- We present an automated workflow for the development of iPSC-derived 3D dorsal forebrain organoids performed by the CellXpress.ai system.
- Functional characterization of organoids was done by recording calcium oscillation activities with the FLIPR Penta instrument. Expected responses to benchmarking compounds were shown.
- Morphological characterization done by imaging demonstrated the presence of neurons and astrocytes, by TuJ1 and GAFP staining, also the presence of progenitor cells positive for SOX2, which decreased during the continuous culture.