3-D Midbrain Floor Plate Model for Differentiation of PSC-derived Dopaminergic Neurons

Richard Josephson, Jonathan Sagal, Soojung Shin, Michael Derr, David T. Kuninger[1]
Thermo Fisher Scientific, Frederick, MD

ABSTRACT

Accurate in vitro modeling of neurological diseases requires multiple cell types of the brain to interact and develop toward mature functionality. When human pluripotent stem cells (PSC) undergo neural differentiation in 3-D, self-organization of progeny cells results in organoids with brain-like structures and functions that are not observed in 2-D culture. However, the increased complexity of neural organoids often comes with the costs of low throughput and poor reproducibility. Disease models for drug discovery may therefore have to temper self-organized complexity with inductive specification of desired cell types.

To model Parkinsons Disease (PD), we have explored methods for differentiation of human PSC to midbrain dopaminergic (DA) neurons in 3-D. In order to compare physical influences on organoid structure we have used a single set of media previously optimized for Midbrain Floor Plate specification, expansion, and maturation in 2-D culture. This media kit worked to specify Floor Plate and differentiate dopaminergic neurons in suspension culture, although the time requirements for specification and differentiation in 3-D could be significantly reduced (please see poster 433.17 on Oct 21, 1 P.M. for more information).

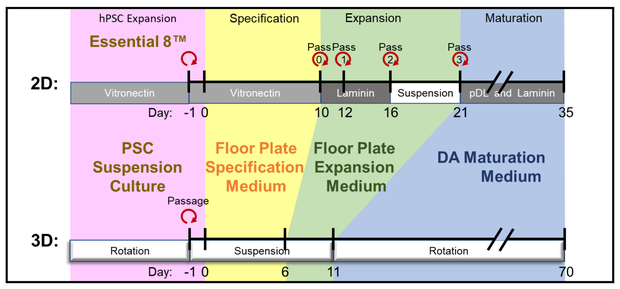
We tested physical parameters including rotation vs. static suspension, and encapsulation within extracellular matrix (ECM) vs. dilute addition of ECM molecules to the medium. Surprisingly, static suspension cultures with addition of dilute ECM could reproduce some of the known benefits of ECM encapsulation, such as morphological complexity and faster neuronal maturation, without the difficulty and low throughput of encapsulation methods.

To apply these promising steps toward a reproducible 3-D model for Parkinsons Disease, we have generated Midbrain Organoids from an iPSC line engineered via CRISPR to carry the PD-linked A30P mutation in alpha-synuclein (SNCA) and an unaltered wildtype line (WT) from the same editing process. We are testing the response of mutant and wildtype organoids to oxidative stressors.

INTRODUCTION

To be useful for drug screening, in vitro disease models must be reproducibly generated at large scale. As a first step toward this goal we tested whether 3-D organoid models of the ventral midbrain could be produced using the simple and scalable Gibco™ PSC Dopaminergic Differentiation Kit. The increased complexity of organoid models has been reported to depend upon cell interactions with basement membrane. We explored methods of organoid formation with or without added extracellular matrix (ECM) for effects upon complexity and neuronal maturation.

Figure 1. Workflow for Floor Plate Specification and Maturation in Suspension Culture



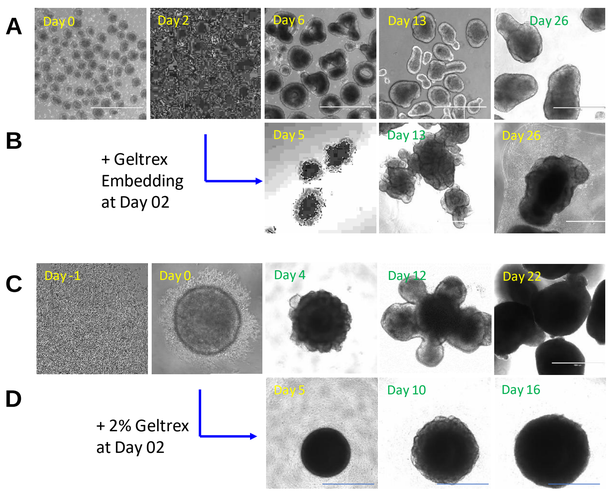
Schematic of the Floor Plate derivation process using the three media in the Gibco™ PSC Dopaminergic Differentiation Kit.

2-D specification with the Kit occurs in attached culture, followed by multiple passages in Expansion Medium until day 21. Floor Plate cells are then passaged onto pDL/laminin differentiation of dopaminergic neurons up to day 35.

The method of 3-D Midbrain Organoid formation begins with PSC in rotating suspension culture. These are dissociated and seeded into suspension culture for Floor Plate Specification, then changed into Expansion Medium and Maturation Medium while in suspension without further passaging. The duration of Specification and Expansion can be shortened for 3-D culture with equivalent expression of Floor Plate markers.

RESULTS

Figure 2. Organoid Formation is Boosted by ECM



A) Floor plate specification and expansion of hPSC in rotating suspension culture without ECM. Numbering of days and passages correspond to the schematic in Figure 1.

B) ECM encapsulation at Day 2 in 50% Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Gibco™) during floor plate specification in rotating suspension.

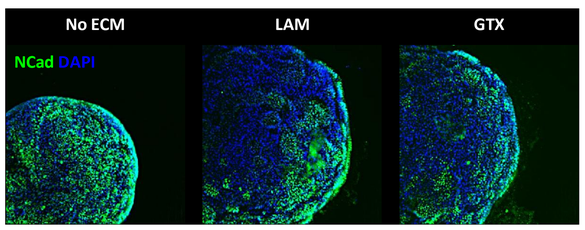
C) Static suspension culture for floor plate specification and expansion in Nunclon™ Sphera™ U-bottom microwells without ECM. Spheroids are transferred to rotating culture for maturation.

D) Static suspension culture in microwells using Floor Plate Specification Medium supplemented with 2% Geltrex™ at Day 2. All other medium changes remain as above.

Scale bars = 1000 microns.

- **ECM encapsulation or U-bottom microplates change the morphology and complexity of Midbrain Floor Plate organoids.**

Figure 3. Added ECM Supports Prominent Rosette-like Structures in Early Organoids



Static microwell organoids after specification by Method C (No ECM), supplemented at Day 2 with 200 µg/mL Laminin (LAM) or with 2% Geltrex™ (GTX). Organoids were fixed on Day 7 and stained with N-Cadherin antibody and DAPI. Maximal intensity projections of 8 micron optical sections imaged on the CellInsight™ CX7 LZR High Content Analysis Platform.

- **ECM addition during Floor Plate specification alters the architecture of microwell organoids.**

Figure 4. ECM encapsulation and U-bottom microplates increase organoid complexity and yield of dopaminergic neurons

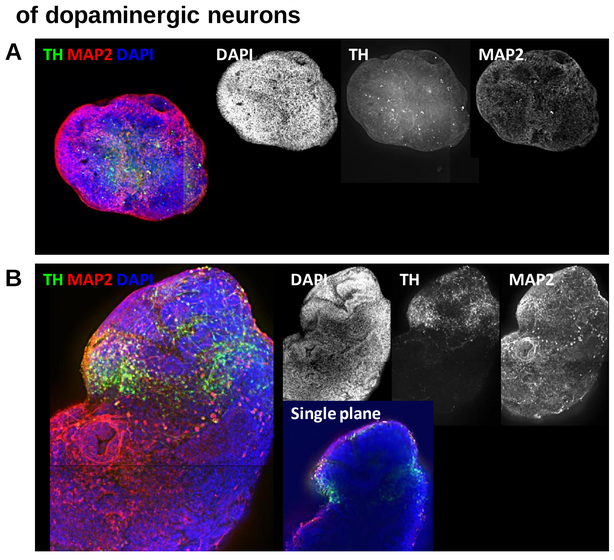
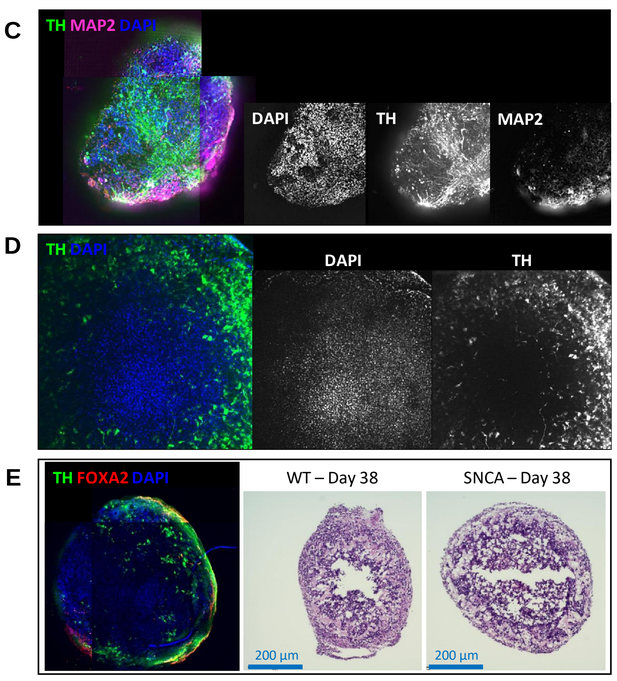


Figure 4 cont… ECM Promotes Organoid Complexity and TH+ Neuron Differentiation



A) Rotating suspension organoid (Method A) at day 19 (early maturation). Maximal intensity projection of MAP2 and tyrosine hydroxylase (TH) antibody staining imaged on the CellInsight™ CX7 LZR High Content Analysis Platform.

B) Encapsulated organoid (Method B) at day 19. Maximal intensity projection of MAP2 and TH antibodies.

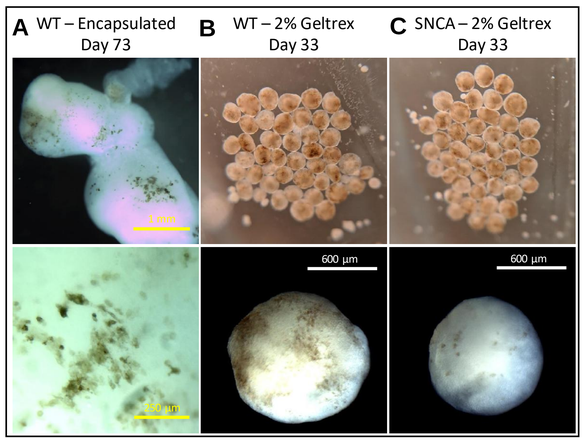
C) Microwell organoid (Method C) at day 19. Maximal intensity projection of MAP2 and TH.

D) 23 day microwell organoid specified in the presence of 2% Geltrex™ at Day 2 (Method D). Maximal intensity projection of TH antibody.

E) Hematoxylin/Eosin stained sections of 5 week old Microwell organoids specified in 2% Geltrex™. Staining reveals thick bands of cells at the outside of the organoids surrounding a dense core of degraded cells.

- **Midbrain Organoids formed with ECM or Microwells have a more complex epithelial morphology and earlier outgrowth of dopaminergic neurons.**

Figure 5. ECM Promotes Maturation of Floor Plate Organoids



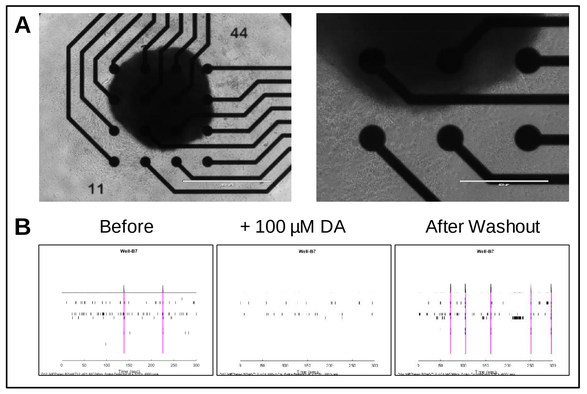
A) Encapsulated organoid (Method B) imaged at day 73 without stains or dyes. Reddish-brown color suggests presence of Neuromelanin, a pigment that is a by-product of Dopamine synthesis (Jo et al., 2014 Cell Stem Cell 19(2):248-257.)

B) Microwell organoids at day 33 specified in 2% Geltrex™ (Method D) from a SNCA Wildtype CRISPR ipsc line.

C) Microwell organoids at day 33 specified in 2% Geltrex™ (Method D) from a SNCA A30P mutant CRISPR ipsc line.

- **Earlier detection of Neuromelanin in microwell organoids with dilute Geltrex™ suggests more rapid maturation of dopaminergic neurons.**

Figure 6. Floor Plate Organoids Mature Functionally

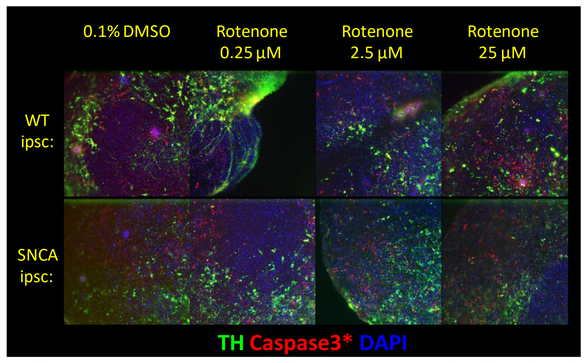


A) Microwell organoid specified in 2% Geltrex™ and cultured in suspension until day 21 and in attached culture on a pDL/laminin coated MEA for 14 days.

B) Raster plots of MEA recordings from the plated microwell organoid above. Each plot shows 300 seconds of activity, first in Maturation Medium, second after addition of 100 µM Dopamine, and third following washout of the dopamine. Vertical pink bars indicate detected network bursts.

- **Midbrain organoids formed in multiwells with dilute Geltrex™ can produce co-ordinated dopaminergic activity in as little as five weeks.**

Figure 7. Modeling Oxidative Stress in Floor Plate Organoids



Oxidative stress test on 5-week Midbrain Organoids derived in multiwells with dilute Geltrex™ from two CRISPR-engineered iPSC lines. Organoids were treated with Rotenone for 20 hours while in rotating suspension. Treated organoids were fixed and serially permeabilized with methanol and Triton X-100, then stained for Active Caspase3 and TH. Wholemount organoids were then cleared with CytoVista™ 3-D Cell Culture Clearing Reagent and imaged on the CellInsight™ CX7. Quantitation of Active Caspase3 staining in TH+ neurons can be performed to measure stress sensitivity.

- **Midbrain organoids can be generated from disease-relevant iPSC lines and used to model responses to environmental stresses.**

CONCLUSIONS

- Midbrain organoids formed in ECM or microwells show greater complexity than organoids in free suspension.
- ECM or microwells promote increased differentiation of dopaminergic neurons within organoids.
- The combination of microwells and dilute ECM during Floor Plate specification contributes to earlier maturation and functional activity of dopaminergic neurons.
- This combined ECM and microwell method offers higher throughput than encapsulation methods for generation of disease-model brain organoids.

TRADEMARKS/LICENSING