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Fabrication of fluid-walled dumbbells and generation of the human corticostriatal pathway

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We use this protocol and it's working

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Abstract

This protocol described the generation of fluid-walled dumbbells for culturing of induced pluripotent stem cell-derived neurons with directional connectivity so as to recapitulate the human corticostriatal connectivity. The fluid-walled microfluidic technology was first reported by **Walsh *et al.*, 2017** and subsequently shown to be **adaptable for cell culture**.

Materials

Reagents:

- Accutase (Stem Cell Tecnologies, CAT# 07920)
- B-27™ Plus Supplement (50X) (ThermoFisher Scientific, CAT# A3582801)
- β-Mercaptoethanol (ThermoFisher Scientific, CAT# 21985023)
- DMEM/F12 basal medium (ThermoFisher Scientific, CAT# 11320033)
- DMEM/F-12, GlutaMAX™ supplement (ThermoFisher Scientific, CAT# 10565018)
- Fluorocarbon 40 (FC40) (iotaSciences Ltd, CAS# 51142-49-5)
- Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix
- Neurobasal (CAT# 21103049)
- MEM Non-Essential Amino Acids Solution (100X) (NEEA) (ThermoFisher Scientific, CAT# 11140050)
- Penicillin-Streptomycin (10,000 U/mL) (ThermoFisher Scientific, CAT# 15140122)
- Poly-D-Lysine (ThermoFisher Scientific, CAT# A3890401)
- ROCK inhibitor Y-27632 (ROCKi) (CAT# 1254)

Equipment:

- In-house Fluid Printer (Iota Science Ltd.)

Preparing cMM solution:

Preparing striatal Neuronal Induction base medium (sNIM):

- DMEM/F12 basal medium
- 1% MEM Non-Essential Amino Acids (NEAA)
- 1% Glutamax
- 1x B27 without vitamin A
- 1% penicillin/Streptomycin (P/S)
- 0.05% β-mercaptoethanol

Troubleshooting

Fabrication of Fluid-Walled Dumbbells

1 **Preparation of petri dishes (Day -7)**

1.1 Pre-coat 6 cm petri dishes with 7 mL of Poly-D-lysine overnight.

1.2 Remove Poly-D-lysine and wash with PBS twice.

1.3 Remove PBS and add a thin layer of neurobasal supplemented with B27.

1.4 Leave to incubate for at least 15 mins.

1.5 Remove neurobasal and add a thin layer of fresh FC40.

1.6 Jet an array of 3*7 dumbbells (3*3 cm) using an in-house fluid printer.

Note

G-Code to jet-print the microfluid-walled dumbbells can be found **here**.

1.7 Coat each dumbbell with 2 µl of 0.46 mg/ml of Geltrex overnight.

Note

Coat dumbbells as soon as possible to avoid evaporation of FC40 which might cause collapse/merging of fluid walls. If working with many dishes, perform **steps 1.6 and 1.7.** in smaller batches.

Establishment of Human Corticostriatal Pathway

2 Day -05: Plating Cortical Neurons (CNs)

2.1 *Preparing media & spinning tubes for CNs plating*

2.1.1. On the day of, before starting, add Rock inhibitor (ROCKi) (1:1000) to cMM and pre-warm this media in the water bath.

2.1.2. Add 9 mL of Neurobasal to a 15 mL falcon (spinning tube) for each vial to be thawed and prewarm.

2.2 *Replating of adherent D32 post-puromycin selection CNs*

2.2.1. Aspirate media from CNs wells and rinse with 1 mL PBS.

2.2.2. Aspirate PBS and add 1 mL of room temperature Accutase per well of 6-well plate.

2.2.3. Incubate at 37ºC for ~5 minutes, until cells detach.

2.2.4. Collect all of the well contents and add to the pre-warmed spinning falcon.

2.2.5. Spin at 350g for 5 minutes.

2.2.6. Resuspend cells in the pre-warmed cMM supplemented with ROCKi (1:1000) and count the cell number using either the cell counter or a haemocytometer.

2.2.7. Calculate the appropriate dilution such that CNs are to be deposited into the cortical chambers of all dumbbells at the replating density of 13,000 cells/dumbbell in 1 µl of cell suspension.

2.2.8. Add 3 µl of cMM into the striatal chamber.

Note

The volume difference on **step 2.2.8.** creates a positive fluid pressure gradient toward the cortical chamber, restricting movement of CN cell bodies away from the chamber.

3 Day -03: Transduction of CNs with LV-Ngn2-GFP

3.1

Transduce CNs in the cortical chamber with LV-Ngn2-GFP, 50 µl of viral stock/2 µl.

3.2

Add 2 µl of fresh cortical media (without lentiviruses) into the striatal chamber.

4 Day 0: Plating of Day 16 MSN progenitors

4.1 Prepare sMM supplemented with ROCKi (1:1000) and pre-warm this media in the water bath.

4.2 Add 9 mL of Neurobasal to a 15 mL falcon (spinning tube) for each vial to be thawed and prewarm.

4.3 Remove media from both chambers.

4.4 Thaw cryovial containing MSN progenitors in water bath until only a small component remains frozen.

Note

Swirl the vial to thaw the MSN progenitors and check frequently to ensure the vial do not get completely thawed while in the water bath.

Be very careful to wipe down the vial with ethanol after removing from the water bath, and try to not place lid underwater to remove the risk of contamination.

4.5 Carefully transfer contents of cryovial to pre-warmed spinning tubes.

4.6 Centrifuge at 350g for 5 min.

4.7 Aspirate media from cell pellet in spinning falcon and replace with 1 mL sMM1 + Rocki, slowly and gently resuspending the pellet.

4.8 Count the cell number using either the cell counter or a haemocytometer.

4.9 Calculate the appropriate dilution such that MSN progenitors are to be deposited into the striatal chamber of all dumbbells at the replating density of 13,000 cells/dumbbell in 1 µl of cell suspension.

Note

For different culturing condition, medium from the striatal chamber is removed and replaced with either 1 µl of either MSN cell suspension (13,000 cells/dumbbell) or standard cortical media (i.e. cMM) or cortical media supplemented with 10-fold BDNF (i.e. cMM with 100 ng/ml BDNF).

4.10 Add 3 µl of cMM into the cortical chamber.

5 **Day 2 – media change**

5.1 Remove 2 µL (i.e. half) of media from each chamber.

5.2 Add 2 µL (i.e. half) of fresh cMM and sMM to the cortical and striatal chamber, respectively.

6 **Day 4, 6 and 8 – media change**
Perform similar media change at these days.