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Localised axotomy of human Cortical Neurons (CNs) from induced pluripotent stem cells (iPSCs)

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We use this protocol and it's working

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Abstract

This protocol describes the process followed to perform localised axotomy of iPSC-derived human cortical axons cultured within fluid-walled dumbbells in 6 cm TCT-treated Petri dishes. A similar protocol was first described by **Soitu et al., 2020** to perform wounds assays in monolayer cell cultures.

Materials

Reagents:

- B-27™ Supplement (50X), serum free (ThermoFisher Scientific, CAT# 17504044)
- FC40 (iotaSciences Ltd, CAS# 51142-49-5)
- Neurobasal (ThermoFisher Scientific, CAT#2113049)
- Phosphate-buffered saline, pH 7.4 (PBS) (Life Technologies, CAT# 10010056)

Equipment:

- In-house Fluid Printer (iotaSciences Ltd.)

Preparation of coating-prep medium:

- Neurobasal
- 1x B-27 supplement

Troubleshooting

Localised axotomy assay

1 On Day 20, remove the FC40 overlay from the Petri dish where human Cortical Neurons (CNs) were cultured inside fluid-walled dumbbells.

2 Gently add ~2 ml of coating-prep medium (see **Materials**) to destroy every fluid wall without peeling cells off the substrate.

3 Remove medium and wash with PBS twice. **Be careful with cells peeling.**

4 After last wash, add ~5 ml of fresh medium (Neurobasal supplemented with B27).

Note

Cells can now be stored in incubator for several minutes if needed.

5 Equip the fluid printer with a 1 mL glass syringe filled with medium.

6 Place dish into the fluid printer.

7 Fluid printer automatically perform axotomy by means of a submerged medium jet. Such jet is held at a fixed height above cells and it is moved around the dish by the printer traverse to cross perpendicularly across axon bundles at the midpoint.

Key parameters:

- jet height =300 µm
- jet flow rate = 480 µl/min
- traverse speed = 960 mm/min

Note

Parameters must be finely tuned, depending on cells maturation and concentration.

Fluid printer is controlled by scripts written in G-code.

8 Remove medium and overlay fresh FC40.

9 New fluid-walled dumbbells can be fabricated around axotomized cultures following **steps 1.5 and 1.6** in **Protocol: Fabrication of fluid-walled dumbbells and generation of the human corticostriatal pathway**

Live-imaging of axonal regeneration

10 On DIV 0, fluorescent live images of all dumbbell conduits were taken just prior to CNs replating to serve as initial normalising timepoint.

11 Images were taken on a digital SLR camera (Nikon D7100 DSLR) connected to an epi-fluorescence microscope (Olympus IX53; 1.25×, 4×, 10×, 25× objectives) equipped with a translation stage and an overhead illuminator (Olympus IX3 with filters).

12 Medium was changed every other day from now on for the next 20 days.

13 Similar live images were taken again on day 20 prior to axotomy, and subsequently 36, 60, and 84 hours post-axotomy.