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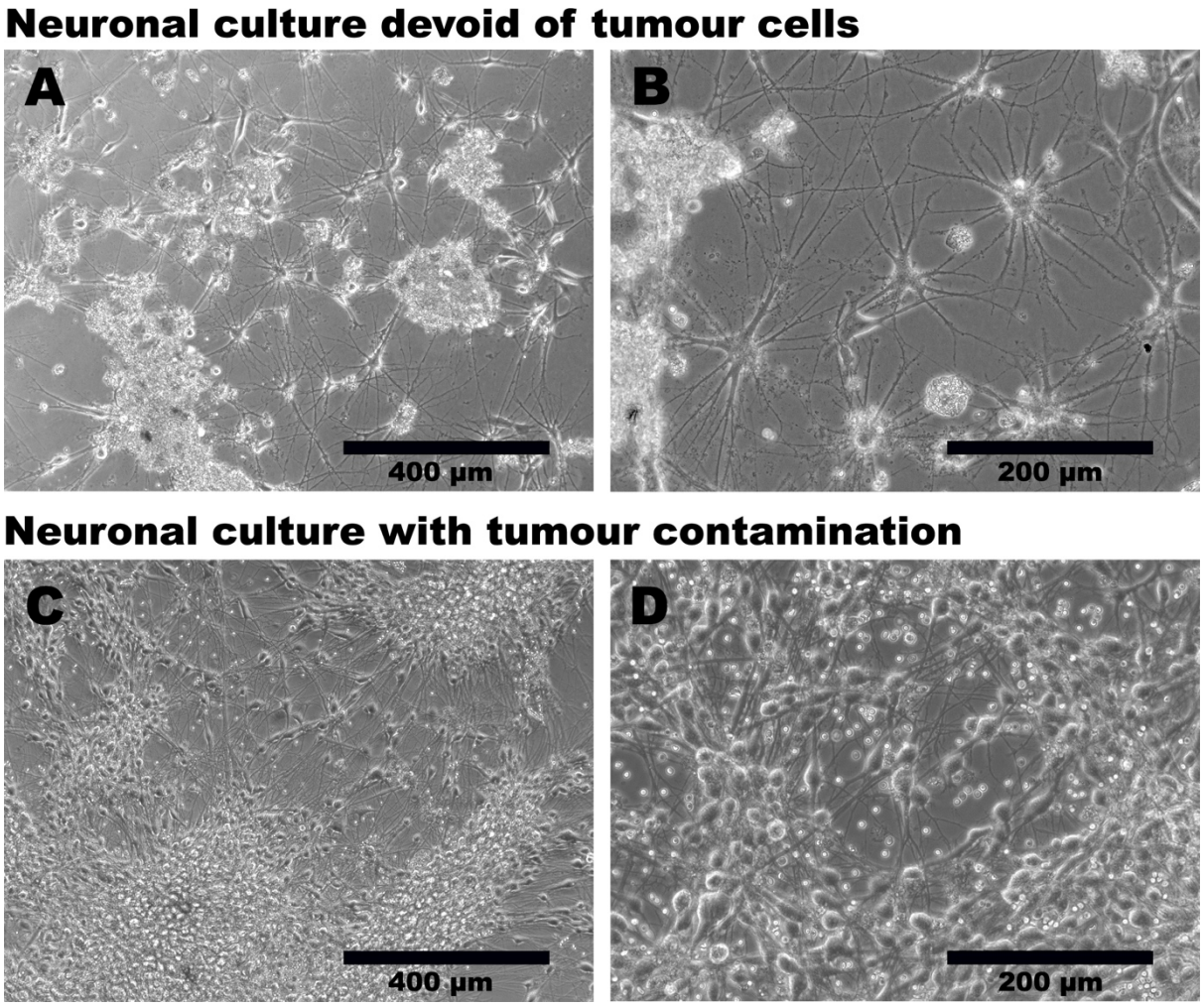
Supplementary information

Routine culture and study of adult human brain cells from neurosurgical specimens

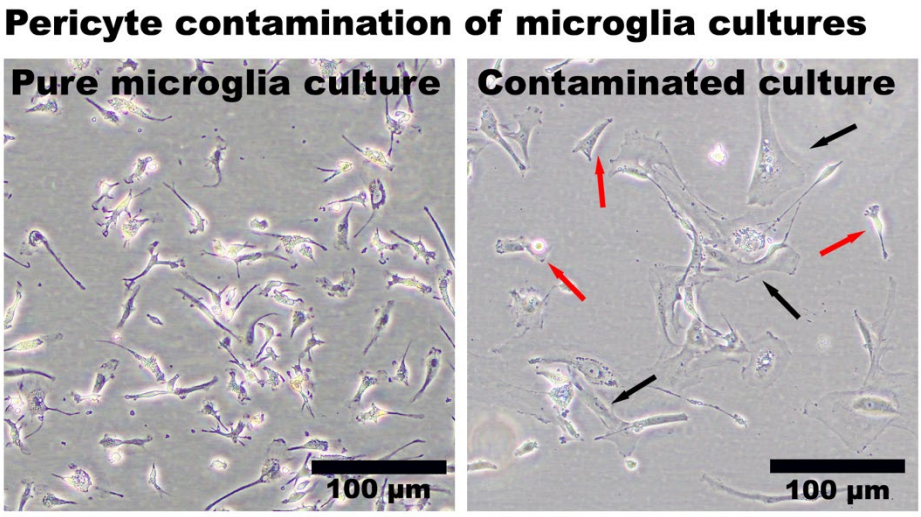
In the format provided by the authors and unedited

Supplementary Figures

Supplementary Figure 1. Photomicrographs of representative neuronal cultures. A-B illustrate a culture that is void of tumour cell contamination and C-D show how a culture appears when there is tumour cell contamination. Scale bars: A and C = 400 µm, B and D = 200 µm. Reproduced from Park et al. 2020, Brain Communications through creative commons license 4.0.



Supplementary Figure 2. Photomicrographs illustrating a high purity microglia culture vs a low purity culture contaminated by pericytes (microglia = Red arrow, pericytes = Black arrows). Scale bar = 100 µm.



Supplementary Tables

Supplementary Table 1: Percentage of $GFAP^+$ astrocytes in mixed glial and neuronal cultures.

Case number	Mixed glial cultures (%)	Neuronal cultures (%)
1	1.2	9.1
2	1.1	14.3
3	1.1	9.1
4		9.3
5		12.9
Mean ± SD	n=3, 1.13 ± 0.05	n=5, 10.9 ± 2.5

Supplementary Table 2: Percentage of $PU1^+$ microglia in mixed glial cultures (n=5)

Case number	Percentage of microglia ($PU1^+$)
1	9.2
2	9.4
3	10.7
4	13.9
5	15.2
Mean ± SD (n=5)	11.7 ± 2.7

Supplementary Table 3: Percentage of $PU1^+$ microglia in enriched microglial cultures (n=14)

Underlying pathology	Case number	Surgery	Percentage of microglia ($PU1^+$)
Intractable Epilepsy (n=5)	1	Temporal lobectomy	91.8
	2	Temporal lobectomy	88.0
	3	Temporal lobectomy	84.0
	4	Temporal lobectomy	82.6
	5	Temporal lobectomy	86.6
		Mean ± SD (n=5)	86.6 ± 3.6
Brain tumours (n=7)	1	Tumour debulking	98.8
	2	Tumour debulking	76.2
	3	Tumour debulking	81.5
	4	Tumour debulking	84.4
	5	Tumour debulking	83.3
	6	Tumour debulking	94.8
	7	Tumour debulking	85.7
		Mean ± SD (n=7)	86.4 ± 7.8
Paediatric cortical malformations (n=2)	1	Cortical resection	74.4
	2	Cortical resection	52.5
		Mean ± SD (n=2)	63.5 ± 15.5

Supplementary information – NPC differentiation

After forming NPC single cell suspension at following steps in 24-C, undertake the following steps to achieve neuronal and astrocytic differentiation of the NPCs.

First, prepare the differentiation media. There are several options, based on your desired outcome.

All neuronal differentiation media contains the following (final concentration is stated below):

1. 1 % (v/v) GlutaMAX® (Gibco; cat. no. 35050-038).
2. 1 % (v/v) Penicillin–streptomycin (Gibco; cat no. 15140).
3. 40 ng/mL Nerve growth factor (NGF, Peprotech, cat. no. 450-01).
4. 40 ng/mL Neurotrophic factor-3 (NT-3, Peprotech, cat. no. 450-03).
5. 40 ng/mL Brain derived neurotrophic factor (BDNF, Peprotech, cat. no. 450-02).

Differentiation media – Option 1: DMEM:F12 + 1 % FBS medium

Dulbecco's Modified Eagle Medium/Nutrient Mix F-12 (DMEM/F12; Invitrogen) supplemented with 1 % (v/v) fetal bovine serum (FBS, Invitrogen).

(a) Widely used to differentiate NSCs/NPCs into neurons and astrocytes.

(b) Results in physiologically active neurons and astrocytes.

(c) However, also supports the growth of pericytes, and in long-term cultures, they will dominate the culture.

Differentiation media – Option 2: DMEM:F12 + B27 + dibutyryl cAMP

Dulbecco's Modified Eagle Medium/Nutrient Mix F-12 (DMEM/F12; Invitrogen) supplemented with 2 % (v/v) B27 with vitamin A (Invitrogen, cat no. 17504-044) and 1 μM dibutyryl cAMP.

(a) The absence of serum limits the growth and proliferation of the pericytes.

(b) Addition of a cAMP analog enhances neuronal differentiation that results in electrically active neurons and astrocytes.

(c) However, there is a slight compromise in cell viability, when compared to the DMEM:F12 1% FBS medium.

Procedure:

1) Collect the single cell suspension obtained from Step 24-C.
2) Dilute the cells using one of the above media to a cell density of 10,000 cells/mL.
3) Plate onto Matrigel-coated tissue culture plates or coverslips (see Reagent Setup – Matrigel) at their designated volumes. (e.g. 96 well plate = 100 μL).
4) Half change media with your designated differentiation media every 2-3 days.

It usually takes 2-3 weeks for the NPCs to differentiate into electrophysiologically-active neurons and astrocytes.