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Microglia differentiation V.1

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We use this protocol and it's working

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Abstract

This protocol has been refined to differentiate microglia cells from hESC adapted to feeder free culture systems as described in the following protocol:

Citation

Douvaras P, Sun B, Wang M, Kruglikov I, Lallos G, Zimmer M, Terrenoire C, Zhang B, Gandy S, Schadt E, Freytes DO, Noggle S, Fossati V
(2017). Directed Differentiation of Human Pluripotent Stem Cells to Microglia..

https://doi.org/10.1016/j.stemcr.2017.04.023 LINK

Protocol Overview

A. Flask Preparation
B. Poly-D-Lysine (PDL) plate coating
C. Media recipes
D. Cell passage and differentiation
E. Microglia precursor isolation and platting for maturation

General Notes

A list of reagents and relevant vendor information can be found in the table listed under the materials tab.

Attachments

PDF

Microglia differenti...

61KB

Materials

Reagent table:

	Item	Vendor	Catalog Number
	DMEM/F12	Gibco	11320082
	Reduced growth factor matrigel	Fisher	CB40230
	T75 Flask	Fisher	07202000
	mTESR	Stem Cell Tech	100-0276
	STEMPRO-34	Gibco	10639011
	BMP4	PeProtech	120-05
	VEGF	PeProtech	100-20
	FGF	PeProtech	100-18B
	SCF	PeProtech	300-07
	IL-3	PeProtech	200-03
	TPO	PeProtech	300-18
	M-CSF	PeProtech	300-25
	FLT-3	PeProtech	300-19
	GM-CSF	PeProtech	300-03
	FLT-3	PeProtech	300-19
	M-CSF	PeProtech	300-25
	TGF-β1	PeProtech	100-21
	IL-34	PeProtech	200-34
	M-CSF	PeProtech	300-25
	Primaria Plates - 6w	Corning	353846
	Primaria Plates - 24w	Corning	353847
	Poly-D-Lysine	Sigma	P6407
	Neurobasal media	Gibco	21103049
	N2 Neuroplex	Gemini	400-163
	GEM21 Neuroplex	Gemini	400-160
	Albumax I	Gibco	11020021
	Sodium Chloride	Fisher	BP35810
	Sodium Pyruvate	Gibco	11360070a
	Glutamax I	Gibco	35050061
	Pen Strep	Glbco	15140122

Flask preparation

1h

1 Thaw matrigel Overnight on ice inside a 4 °C fridge. Chill all flasks, pipettes and materials to be used for flask preparation under the same conditions and keep everything as cold as possible throughout the procedure (do not use a freezer as it can cause the matrigel solution to freeze while platting).

2 Dilute matrigel in cold DMEM/F12 as described by the manufacturer and add into the T75 flasks (~ 6 mL /flask total volume). Incubate at 37 °C for at least 01:00:00 before use. Flasks can be stored in the incubator for several weeks before use.

1h

Poly-D-Lysine (PDL) plate coating

6h

3 Prepare PDL (0.5 µg/ml) in sterile water and add it to each well and incubate 06:00:00 to Overnight at 37 °C. The day after, wash each well 4 times with sterile water to remove excess PDL (at this point the plates are ready to be used for culture - do not let the wells dry at any step as it can cause PDL crystallization (cytotoxic)).

6h

Media preparation

4 The below media recipes are used in the remainder of the protocol:

4.1 **STEP1 media:** mTeSR media + Penicillin-Streptomycin (100U/ml)+ 80ng/ml BMP4

4.2 **STEP2 media:** StemPro-34 SFM media + 2 mM GlutaMAX + 80 ng/ml VEGF, 25 ng/ml FGF + 100 ng/ml SCF

4.3 **STEP3 media:** StemPro-34 SFM media + 50 ng/ml SCF + 50 ng/ml IL-3 + 5 ng/ml TPO + 50 ng/ul M-CSF + 50 ng/ul Flt3

4.4 **STEP4 media:** StemPro-34 SFM media + 50 ng/ml SCF + 50 ng/ml IL-3 + 5 ng/ml TPO + 50 ng/ul M-CSF + 50 ng/ul Flt3

4.5 **Microglia maturation media:** Neurobasal media + N2 Neuroplex (1x final concentration) + GEM21 Neuroplex (1x final concentration) + 20% AlbuMAX I (0.2% final concentration)

+ NaCl (5M) (50mM final concentration) + sodium pyruvate 100x (1x final concentration) + glutaMAX 100x (1x final concentration) + Penicillin-Streptomycin (100U/ml) + 50 ng/ml TGF-β1 + 100 ng/ml IL-34 + 12.5 ng/ml M-CSF

Cell passage and differentiation

5

Note

It is important to start the differentiation from pristine, undifferentiated feeder free cultures. For more details consult: https://doi.org/10.17504/protocols.io.b4mcqu2w

Note

When changing media in the flasks, avoid scraping the bottom surface of the flasks to prevent cell and coating loss.

Manually dissect undifferentiated hPSC colonies and transfer 40-60 small aggregates into a matrigel coated flask.

6 Maintain undifferentiated cells in mTeSR media until undifferentiated colonies reach an average colony size of 1 mm. Usually, 7 mL of media/flask and media change every other day works well to maintain the cells until they reach the right size.

7 STEP1 (days 0-3): Once the hPSC colonies reach the correct size, microglia differentiation is initiated (day 0) by culturing the undifferentiated cells in 6 mL of STEP1 media. STEP1 media should be replaced with fresh media on day 2.

8 STEP2 (days 4-5): Culture cells in 6 mL of STEP2 media. No media change until the next step.

9 STEP3 (days 6-13): Culture cells in 6 mL of STEP3 media. Media should be changed on day 10.

10 STEP4 (days 14-28): Culture cells in 6 mL of STEP4 media. Media should be replaced every 96:00:00 . 4d

Microglia precursor isolation and platting for maturation

4m

11

4m

Note

The timepoint of microglial precursor generation as indicated by the appearance of a large number of floating cells with microglial precursor typical morphology in the supernatant is variable between hPSC cell lines and starts usually around day 28 of differentiation. Microglia precursor differentiation can be validated by FACS analysis (described in another protocol in this same collection). We normally only initiate microglial differentiation from cultures with more than 80% CD11b/CD45 and CD14/CD16 double positive microglial precursors.

Note

If properly maintained with STEP4 media changes every 4 days, cultures in STEP4 are capable of generating microglia precursors for several months (as monitored by FACS). Microglia precursors can be collected every 4 days for microglial maturation as described below.

Note

For media changes in STEP4 on days without collecting microglial precursors for maturation (as described below), we usually try to return microglial precursors after media change. Because microglia precursor cells grow mainly in suspension, we collect ~ 5 mL of microglial containing supernatant in a conical tube, centrifuged at 150 rcf for 00:04:00, and subsequently resuspend the cells in 5 mL of fresh STEP4 media to return to the same flask.

Note

Microglial precursors can be plated on either Primaria plates or Poly-D-Lysine (PDL) coated cell culture plates or glass cover slips. However, based on our experience, platting on primaria plates results in more consistent differentiation to mature microglia.

Once microglia precursor differentiation is validated on day 28 or later, microglia progenitors are collected from STEP4 flasks by transferring the supernatant to a conical tube followed by centrifugation at 150 rcf, Room temperature for 00:04:00.

The cell pellet is resuspended in microglia maturation media and plated at 75k cells/cm2 (Primaria or PDL-coated plates) in microglia maturation media. Media should be changed every 4 days for at least 2 weeks to allow the cells to mature.

Citations

Douvaras P, Sun B, Wang M, Kruglikov I, Lallos G, Zimmer M, Terrenoire C, Zhang B, Gandy S, Schadt E, Freytes DO, Noggle S, Fossati V. Directed Differentiation of Human Pluripotent Stem Cells to Microglia. **https://doi.org/10.1016/j.stemcr.2017.04.023**