The production of monocytes and macrophages from human iPSCs

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Abstract

This protocol is a modification of a previously published method by Wilgenburg et al (PLoS One, 2013) to obtain monocytes and macrophages from induced Pluripotent Stem Cells (iPSCs) lines.

Citation: Katherine Santostefano,Jared Taylor,Naohiro Terada,Mark Wallet The production of monocytes and macrophages from human iPSCs. **protocols.io**

https://www.protocols.io/view/the-production-of-monocytes-and-macrophages-from-h-m8kc9uw

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Before start

1. Order neccessary supplies

Item	Catalog #	Vendor
hES Medium		
KO DMEM	10829-018	Gibco
KO Serum replacement	10828	Gibco
NE Amino Acids	M11-003	PAA
GlutaMAX	35050	Gibco
2-Mercaptoethanol	31350-010	Gibco
bFGF	4114-TC-01M	&D Systems
Pen/Strep	P11-010	PAA
X-VIVO FACTORY base		
X-VIVO 15	BE04-418	Lonza
GlutaMAX	35050	Gibco
2-Mercaptoethanol	31350-010	Gibco
Additional Growth Factors		
M-CSF	300-25	PeproTech
IL-3	200-03	PeproTech
Rock inhibitor: Y-27632 dihydrochloride	1254	Tocris

Other		
Ultra-low attachment 6 well plates	3471	Corning from Appleton Woods/or Fisher Scientific Ltd
hESC-Qualified Matrix Matrigel	354277	Corning
mTeSR1 media	05850	Stem Cell Technologies

2. Prepare solutions

X-VIVO Factory base medium

1. 500mL X-VIVO 15
2. 5mL Glutamax (100X)
3. 500µL 2-ME (1000x)

FACTORY medium complete

1. 25 mL of basic medium
2. 250 µL MCSF / IL-3 cocktail

   Stock MCSF = 20 µg/mL (final = 200 ng/mL)

   Stock IL-3 = 5 µg/mL (final = 50 ng/mL)

3. Pen/Strep

MACROPHAGE DIFFERENTIATION base

1. 500mL DMEM
2. 50mL human AB serum (heat inactivated) – we test multiple vendors and multiple lots from each vendor and buy in bulk. The test is performed using healthy human donor peripheral blood monocytes differentiated in MACROPHAGE DIFFERENTIATION complete medium with different human sera lots. This is critically important as we have found massive variability in the capacity of human sera to support macrophage differentiation. A “good” serum should result in full conversion of monocytes into fried egg appearing macrophages within 7 days.
3. 5mL GlutaMAX
4. Pen/Strep

MACROPHAGE DIFFERENTIATION complete

1. To a 50mL aliquot of the base medium, add 1 ng/mL M-CSF (=5 ul of 10 µg/mL stock solution)

Protocol

Sample

Step 1.

Start with a well of a 10 cm plate with iPS cells grown on Matrigel as follows:

A. Culture of iPSCs/ESCs on Matrigel (hES qualified) coated dishes in a 10 cm dish

NOTES

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Cells require media changes every day starting on Day 1. The maintenance media is orange in color to begin with. The following is a guideline, but you can add more fresh media as necessary

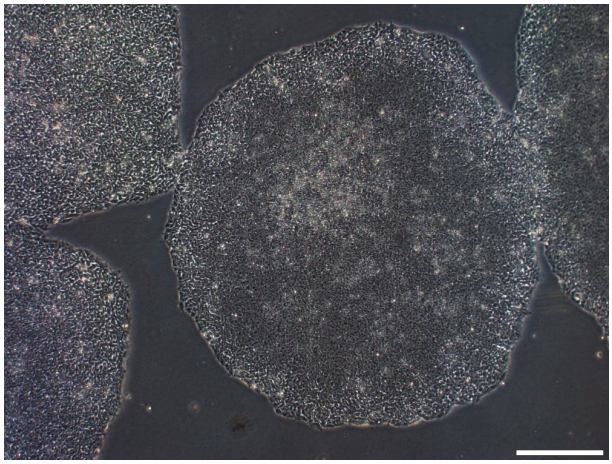
Daily Maintenance

Step 2.

- **Day 0,** Passage iPSCs onto 10 cm Matrigel coated dish in 8 mL mTesR1 media **passage clumps sparsely so that they will have room to grow into large colonies by Day 10** (**See Image 1**)
- **Day 1 Δ media,** add 8 mL fresh media
- **Day 2 Δ media,** add 8 mL fresh media
- **Day 3 Δ media,** add 9 mL fresh media
- **Day 4 Δ media,** add 9 mL fresh media
- **Day 5 Δ media,** add 10 mL fresh media
- **Day 6 Δ media,** add 10 mL fresh media
- **Day 7 Δ media,** add 11 mL fresh media
- **Day 8 Δ media,** add 11 mL fresh media
- **Day 9 Δ media,** add 12 mL fresh media

EXPECTED RESULTS

Image 1: iPSC colonies just before lifting for EB culture.



Setting up Embryoid Bodies (EBs)

Step 3.

Pre-treat iPSCs for 1 hr with 10 µM ROCK inhibitor (Y-27632) to reduce apoptosis of cells that occurs during the scraping step

Setting up Embryoid Bodies (EBs)

Step 4.

Add 2 mL fresh media (+10 µM ROCK inhibitor) to each well of a 6-well non-adherent plate (to pre-wet the wells)

Setting up Embryoid Bodies (EBs)

Step 5.

Use a cell lifter to gently dislodge the colonies, transfer colonies and media gently to a 15 mL conical tube to allow colonies to gravity settle. Aspirate media as soon as large colonies are settled (this will help remove small colonies or single cells)

Setting up Embryoid Bodies (EBs)

Step 6.

Add 12 mL mTeSR1 + ROCK inhibitor to 15 mL conical tube with settled colonies, gently mix and distribute the volume equally among wells of the 6-well plate prepared above (total volume per well = 4 mL mTeSR1 + ROCK inhibitor (10 µM)

Setting up Embryoid Bodies (EBs)

Step 7.

Change ¾ media on Day 2 (tip plate and allow EBs to settle to the bottom edge). Rather than using vacuum aspiration it is easier to remove media using a serological pipet. Replace with 4 mL fresh mTeSR1 + ROCK inhibitor per well

Setting up Embryoid Bodies (EBs)

Step 8.

Collect EBs on Day 4. Transfer EBs and media to 15 mL conical tube(s). Gravity settle EBs, remove

media, wash with plain XVIVO-15 media (no growth factors). (**See Image 2 & 3 for examples of EBs**)

EXPECTED RESULTS

Image 2: EBs after 4 days culture [Note, this is an exceptionally good looking culture. Often, cystic regions will be less prominent, but differentiation still works]

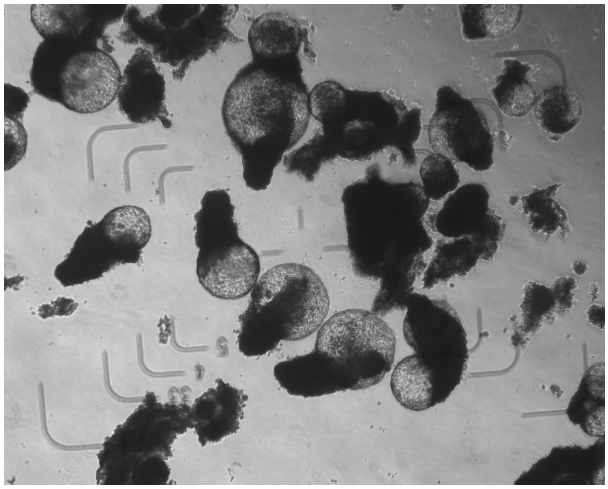
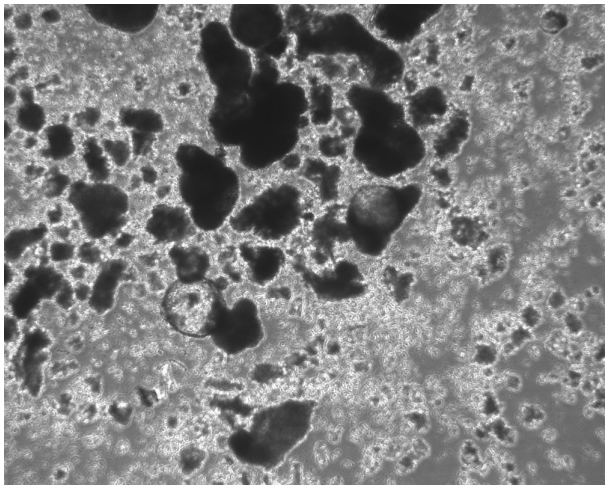


Image 3: Less photogenic EBs that still yielded quality monocytes [Note the small debris because the wash step to remove small cells/debris had not been included in the protocol at this point].



Setting up FACTORIES

Step 9.

Estimate the total number of medium to large EBs formed (this is a judgement call that becomes easier only with experience).

Setting up FACTORIES

Step 10.

Harvest EBs into a 50mL conical tube and allow to settle for a few mins. The large clumps (= good EBs) will sink, whilst individual/dead cells will remain floating. Remove supernatant, add 10ml factory base Repeat settling and wash with Factory base medium.

Setting up FACTORIES

Step 11.

Resuspend EBs in 4 ml factory complete medium for every 20 EBs. Transfer 4 mL EBs + medium into each well of an adherent, regular tissue culture 6-well plate. Aim to end up with around 15 - 20 EBs per well (too many will inhibit monocyte production). If the original stem cell well was dense the EBs may be split between two or more wells.

Setting up FACTORIES

Step 12.

Incubate for several days. If there is a lot of cell debris, then medium may need changing (warm medium, and always leave at least 1mL in the well), but otherwise do 50% approximately every 5 days or when the medium becomes yellow. Total volume can be increased to 6mL.

NOTES

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EBs set up as factories in X-VIVO factory medium may not adhere to plates (most often the EBs adhere, but adherence is weak so medium exchange must be done very gently).

Harvesting monocytes for macrophage differentiation

Step 13.

Monocytes will start to appear in supernatant after 10 – 20 days. (**See Image 4**) The monocyte harvest described below can be performed every 5 days for approximately 4-6 weeks. The first harvest yields the fewest monocytes, but yield tends to increase for harvests 2-5. Total yield from a 6-well plate of factories can reach about 2 million monocytes per harvest.

A. Gently swirl plate then harvest 5ml of the supernatant (leaving 1ml behind) - be careful not to disrupt factory, and always use warm medium, otherwise factory may curl up. (Care is required for XVIVO factories since factories may not have adhered to plate).

B. Count cells, spin at 400g for 5mins, resuspend pellet in MACROPHAGE DIFFERENTIATION medium. Plate at 0.3 x 10^6 per well of 24 well plate, 1mL/well (or equivalent density for other well-sizes; 1 x 10^5 works well for 96 well plates).

C. Cells will adhere within 24 hours, and become spindly over the course of the week.

D. Change 50% of medium at day 3.

E. At day 7 macrophages are ready to use (remove any remaining non-adherent cells – sometimes there is noticeable proliferation). Change 50% medium every 5 -7 days if keeping for longer. (**See Image 5**).

EXPECTED RESULTS

Image 4: monocyte production region within adherent EB after 10 days in complete factory medium [Note, the smooth round cells concentrated near the lower right are monocytes. When leaving these foci of monocyte generation and entering the surrounding medium, the monocytes are more vacuolated and often have small dendrites].

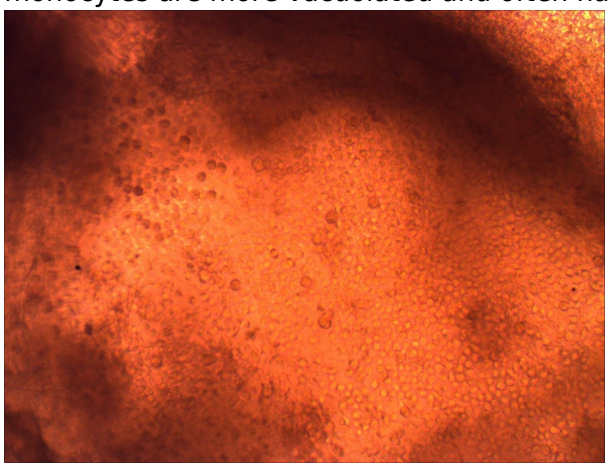

Image 5: iPSC-derived macrophages grown for 7 days in macrophage differentiation complete medium followed by 11 days in macrophages differentiation base medium [Note, cells are fully differentiated at day 7. This culture was part of a trial to determine how long macrophages can be kept in culture. The cells maintained morphology with little evidence of cell death for 5 weeks]