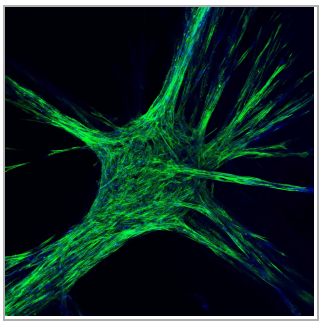

DEC 16, 2022

WORKS FOR ME 1

Generation of 3-dimensional spheroids of human umbilical vein endothelial cells and human amnion-derived mesenchymal stem cells in human platelet lysate-based gels

COMMENTS 0

DOI
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ABSTRACT

Generation of 3-dimensional spheroids containing human umbilical vein endothelial cells and human amnion-derived mesenchymal stem cells. The protocol uses an optimized cell ratio to obtain stable spheroids that can be transferred to human platelet lysate-based gels. Within the gels the spheroids form sprouts and interconnect with each other. After grwoth, the spheroids can be fixed in the gel and stained for confocal microscopy.

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EXTERNAL LINK

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PROTOCOL INTEGER ID

61848

GUIDELINES

Critical steps:
1) Use primary cells at an early passage
2) do not handle the hanging drop culture too roughly, also carefully open/close the incubator door
3) take your time while transferring the spheroids to the gel

MATERIALS TEXT

Human umbilical vein endothelial cells (HUVECs) P0
Human amnion-derived mesenchymal stem cells (hAMSCs) P0
M-199 Medium (Sigma)
FBS (Gibco)
Endothelial cell growth supplement ECGS (Becton Dickinson, stock 20 µg/ml)
Human platelet lysate (Macopharma)
Heparin (Baxalta, 5000 I.E./ml)
Thrombin (Sigma, stock 250 U/ml)
µ-dishes (Ibidi)
Hydrophobic petri dishes 100 mm (Greiner)
1x PBS pH 7.4 (Gibco)
Stem Pro Accutase (Gibco)
Luna Cell Counter slide (Logos Biosystems)
Trypan blue solution (Sigma)
Aprotinin (Sigma, stock 3500 U/ml)
T-25 flask (Greiner)
15-ml falcon tube (Greiner)

Equipment:
Luna Automated Cell Counter (Logos Biosystems)
Steri Cycle CO_2 Incubator (Thermo)
Rotana 460R centrifuge (Hettich)

BEFORE STARTING

Isolate the human umbilical vein endothelial cells and human amnion-derived mesenchymal stem cells and culture them in their corresponding medium until 70-80% confluency (passage 0). https://doi.org/10.3389/fbioe.2019.00338

Harvesting of the cells

1 Aspirate the medium and wash the cells with 1x PBS

2 Add 300 µl accutase to a T-25 flask and incubate at 37 °C, 5% CO_2 for 3-5 minutes

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3 Resuspend the cells in 10 ml PBS and transfer the cell suspension to a 15-ml falcon tube

4 Centrifuge at 500*g* for 5 minutes at 20 °C

5 Aspirate the supernatant and dissolve the pellet in 10 ml M-199 medium (serum-free)

6 Count the cells using a Luna Cell Counter

Hanging drop

7 Prepare cell suspension for hanging drops

5000 cells in 25 µl drop

For 40 spheroids:

Kultur	HUVEC	hAMSC	Medium
10% HUVECs + 90% hAMSCs	2*10^4	1.8*10^5	1000 µl

For 10 ml medium:

Reagent	Stock solution	End concentration	Volume for 10 ml
M-199			8 ml
FBS	100%	20%	2 ml
ECGS	10 mg/ml	10 µg/ml	10 µl
Heparin	5000 I.E./ml	15 I.E./ml	30 µl

8 Pipette 25 µl drops on the inner side of the lid of a pertridish (hydrophobic)

9 Carefully flip the lid

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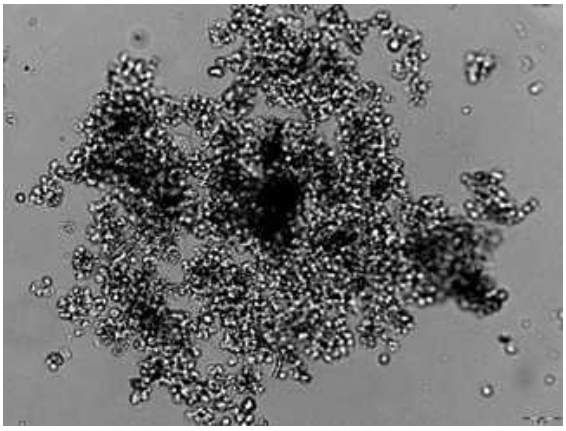
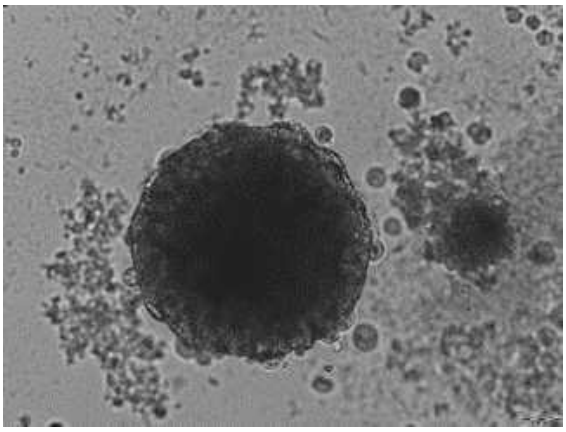
10 Put the lid on the bottom of the petridish filled with 20 ml PBS

11 Incubate for 48 hours at 37 °C, 5% CO_2



Day1



Day2

Culturing of the spheroids in HPL-gels

12 Prepare gel mix (500 µl/µ-dish)

Gel mix for 2 gels

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Reagent	Volume for 1000 µl
HPL	200 µl
Aprotinin	58 µl
M-199	741 µl
ECGS	1 µl

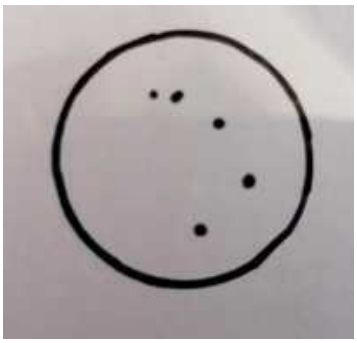
Aprotinin is added to prevent fibrinolysis.

13 Mix 500 µl Gel mix with 100 µl thrombin

14 Carefully pipette the gel mix on the window of the µ-dish (avoid bubbles)

15 Polymerize the gels at 37 °C for at least 20 minutes

16 Pick a spheroid with a 200 µl pipette tip (avoid transferring too much medium) and place the spheroid on the gel (positioning according to the picture)



17 Repeat step 16 until 5 spheroids are transferred to the gel

18 Put the µ-dish in a bigger pertridish with PBS (to prevent dehydration of the gels) and incubate at 37 °C, 5% CO_2 overnight

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19 After 24 hours sprouts are visible

20 Add medium to the gel (500 µl/gel), pipette carefully at the border of the dish

For 2 ml medium:

Reagent	Stock solution	End concentration	Volume for 2 ml
M-199			1.6 ml
HPL	100%	20%	400 µl
ECGS	10 mg/ml	10 µg/ml	2 µl

21 Incubate at 37 °C, 5% CO_2

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