Protocol for controlled modeling of human epiblast and amnion development using stem cells

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Method Article

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Abstract

Due to the inaccessibility of post-implantation human embryos and the restriction on in-vitro fertilization (IVF) embryos cultured beyond 14 days, the knowledge of early post-implantation human embryogenesis remains extremely limited. Recently, we have developed a microfluidic in-vitro platform, based on human pluripotent stem cells (hPSCs), which is capable of recapitulating several key developmental landmarks of early human post-implantation embryonic development, including lumenogenesis of the epiblast (EPI), amniogenesis, and specification of primordial germ cells (PGCs) and of primitive streak (PS) cells. Given its controllability and reproducibility, the microfluidic platform provides a powerful experimental platform to advance knowledge of human embryology and reproduction. This protocol describes the preparation of the microfluidic device and its implementation for modeling human post-implantation epiblast and amnion development using hPSCs.

Introduction

Reagents

1. A positive silicon wafer mold made using Deep Reactive Ion Etching (DRIE)

2. Polydimethylsiloxane (PDMS) (Dow SYLGARD™ 184)

3. Coverslip (Fisher Scientific, Cat. 22-266-973)

4. mTeSR™1 (STEMCELL Technologies, Cat. 85850)

5. Geltrex (Thermo Fisher Scientific, Cat. A1413202)

6. Y27632 (Tocris, Cat. 1254)

7. ACCUTASE™ (Sigma-Aldrich, Cat. A6964)

8. Essential 6 Medium (Thermo Fisher Scientific, Cat. A1516401)

9. FGF-Basic (AA 1-155) (Thermo Fisher Scientific, Cat. PHG0266)

10. BMP4 (R&D Systems, Cat. 314-BP-050)

11. ACTIVIN (R&D Systems, Cat. 338-AC-050)

12. NOGGIN (R&D Systems, Cat. 6057-NG-025)

13. IWP2 (Tocris, Cat. 3533)

Equipment

Procedure

Maintain hPSCs in mTeSR™1 on 1% Geltrex-coated 6-well tissue culture plates in a cell incubator (37°C, 5% CO_2).

Day 1:

1. Mix the curing agent and elastomer of PDMS at a 1:10 ratio, and degas the mixture in a desiccator for 40 mins.

2. Cast PDMS mixture onto the silicon mold with designed features, and bake at 110 °C for 40 mins. PDMS layers can be stored in a clean zipper bag, and good to use for a long time.

Day 2:

3. Punch medium loading wells (8mm in diameter) and gel loading ports (1.2mm in diameter) using Harris Uni-Core punch tools (Ted Pella).

4. Cut off individual devices, clean the PDMS layers with Scotch tapes to remove particles and debris, then wash coverslips with ethanol in a sonicator for 60 mins.

5. Bake coverslips at 110 °C for 20 mins.

6. Treat the PDMS layers and coverslips with oxygen plasma for 30 secs, and bond together, then bake at 80°C overnight.

Critical: When bonding PDMS layers with coverslips, slightly apply pressure for 20 secs. 80°C overnight is needed to restore the hydrophobicity of the devices, critical for the gel injection step.

Day 3:

7. Expose the devices to UV light for 30 mins in a cell culture hood.

8. Prepare a humid chamber by putting a 35 mm Petri dish filled with sterile DI water in a 150 mm Petri dish. Humid chamber is to prevent the medium evaporating too fast.

9. Dilute Geltrex with cold mTeSR™1 to achieve a final concentration of ~8 mg/ml.

Critical: Keep Geltrex on ice all the time, and mTeSR™1 needs to be cold (0 - 4 °C). Geltrex is very sensitive to temperature, partially cured Geltrex will result in experimental failure.

10. Inject diluted Geltrex into a gel loading port to fill the gel channel.

Critical: Injection should be very gentle, so that the Geltrex is only confined in the central channel by surface tension. Geltrex does not need to fill the other gel loading port.

11. Allow the Geltrex to cure for 10 mins in the humid chamber in a cell incubator. During incubation, Geltrex forms concave pockets between supporting posts. Due to the batch variations and environmental factors, the operator needs to check the pocket size every 1-2 mins after 5 mins incubation.

12. When the pockets reach desired size (100-120 μm in diameter), fill the side channels with mTeSR™1 medium by gently applying vacuum with a rubber dropper bulb. 180 μL per well for the cell seeding channel, and 140 μL per well for the induction channel is recommend.

13. Incubate the devices filled with mTeSR™1 in a cell incubator for 18-24 hours to stabilized the Geltrex and remove trapped bubbles.

Day 4:

14. Dissociate human pluripotent stem cell (hPSC) into single cells by incubating with Accutase at 37 °C for 10 mins, and resuspend the cells in mTeSR™1 containing 10 μM Y27632, at a concentration of 8×10^6 cells mL^{-1}. Keep the cell suspension on ice.

15. Empty all the wells of the devices with a vacuum aspirator.

Critical: Just empty the wells but not the channels by placing the vacuum aspirator away from the channel inlets.

16. Introduce 10μL hPSC suspension into the cell loading channel, then allow to sediment into the gel pockets by tilting the device by 90° for 10 mins. It is recommended after 5 mins, gently pipette the 10μL hPSC suspension in the well to stir up the cells.

17. Fill the devices with mTeSR™1 containing 10 μM Y27632. 200 μL per well for the cell seeding channel, and 160 μL per well for the induction channel is recommend.

Day 5:

18. After 18 hours incubation, hPSC clusters will form within the gel pockets (t = 0 h).

Note: Unless otherwise noted, all medium reservoirs and both cell loading and induction channels are filled with basal medium (BM) comprised of Essential 6 Medium and FGF2 (20 ng mL^{-1}) from t = 0 h onwards and are replenished daily.

To generate epiblast-like cysts, BM is injected in all medium reservoirs from t = 0 h onwards.

To generate posteriorized embryonic-like sacs, BMP4 (50 ng mL^{-1}) is supplemented into BM in the induction channel from t = 0 h onwards.

To generate anteriorized embryonic-like sacs, in addition to BMP4 (50 ng mL^{-1}) supplemented into BM in the induction channel, NOGGIN (50 ng mL^{-1}) and IWP2 (5 μM in DMSO) are supplemented into BM in the

cell loading channel from t = 0 h onwards.

To examine the development of posteriorized mesoderm-like cells, from t = 0 h onwards, BMP4 (50 ng mL^{-1}) is supplemented into BM in the cell loading channel, and BM is loaded into the induction channel.

To examine the development of anteriorized mesoderm-like cells, in addition to BMP4 (50 ng mL^{-1}) supplemented into BM in the cell loading channel, ACTIVIN A (50 ng mL^{-1}) is added into BM in the induction channel.

Troubleshooting

Troubleshooting

1. Geltrex leaks to side channels

Injection force is too strong.

Time of 80°C baking after PDMS and coverslips bonding is short.

Geltrex has partially cured before injection.

2. Geltrex does not form pockets after incubation or breaks during cell seeding

Geltrex concentration is low. The dilution of Geltrex needs to be optimized for different batches (recommended range: 55 % to 70%).

3. hPSC clusters are too small

Cell seeding time needs to be optimized for specific hPSC lines and culture conditions.

Time Taken

Anticipated Results

Please find the associated publication.

References

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