Protocol for liver organoid formation from mouse embryonic liver cells using MatriMix (511)

1. Materials

- Pregnant mouse (ICR, 12th day of pregnancy)
- Stereo microscope
- Lab gloves, small dissection scissors, tweezers
- Sterilized Petri dish (φ60 × 14 mm, φ40 × 14 mm)
- 1 ml syringe x 2, 18 G needle x 2, 27 G needle x 2
- 40 μm filter (Falcon #352340)
- Cell scraper
- Cell culture plate (12-well, CORNING #3513)
- Accutase (Innovative Cell Technologies #AT104)
- Advanced DMEM/F12 (Invitrogen #12634-010)
- Fetal bovine serum
- Penicillin/Streptomycin (Thermo Fisher #15070-063)
- GlutaMAX™ Supplement (Thermo Fisher #35050061)
- HEPES (1 M) (Thermofisher #15630106)
- Primocin (Invivogen #ant-pm-1)
- FGF7 (R&D Systems #251-KG)
- BMP4 (R&D Systems #314-BP)
- Activin A (R&D Systems #338-AC)
- N-acetyl-L-Cysteine (FUJIFILM Wako #013-05133)
- [Leu15]-Gastrin I human (Sigma #G9145)
- EGF (PeproTech #AF-100-15)
- FGF10 (PeproTech #100-26)
- A83-01 (R&D Systems #2939)
- DAPT (Adipogen life science AG-CR1-0016-M005)
- Dexamethasone (FUJIFILM Wako #194561)
- R-spondin1 (R&D Systems #4645-RS-025)
- HGF, Human, Recombinant (PeproTech #100-39H)
- VEGF (R&D Systems #293-VE-010)
- bFGF (R&D Systems #233-FB)
- MatriMix511 (AMSBIO #AMS.899-001)

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2. Preparation of culture medium

2-1. Preparation of growth factors

- FGF7 : Dilute in Advanced DMEM/F12 to 10 µg/ml. Store working aliquots at -20 ˚C.
- BMP4 : Dilute in 4 mM HCl to 50 µg/ml. Store working aliquots at -20 ˚C.
- Activin A : Dilute in 4 mM HCl to 100 µg/ml. Store working aliquots at -20 ˚C.
- N-Acetyl cysteine : Dilute in sterile purified water to 100 mM. Store working aliquots at -20 ˚C.
- [Leu15]-Gastrin I : Dilute in sterile purified water to 10 mM. Store working aliquots at -20 ˚C.
- EGF : Dilute in sterile purified water to 100 µg/ml. Store working aliquots at -20 ˚C.
- FGF10 : Dilute in Advanced DMEM/F12 to 100 µg/ml. Store working aliquots at -20 ˚C.
- A83-01 : Dilute in DMSO to 50 mM. Store working aliquots at -20 ˚C.
- DAPT : Dilute in DMSO to 50 mM. Store working aliquots at -20 ˚C.
- Dexamethasone : Dilute in DMSO to 500 mM. Store working aliquots at -20 ˚C.
- R-spondin 1 : Dilute in sterile PBS (-) to 100 µg/ml. Store working aliquots at -20 ˚C.
- HGF : Dilute in sterile purified water to 0.1 mg/ml. Store working aliquots at -20 ˚C.
- VEGF : Dilute in Advanced DMEM/F12 to 50 µg/ml. Store working aliquots at -20 ˚C.
- bFGF : Dilute in Advanced DMEM/F12 to 100 µg/ml. Store working aliquots at -20 ˚C.

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2-2. Prepare medium using the following culture media composition

Basal media (500 ml)

Media and Growth factor	Addition amount	final concentration
Advanced DMEM/F12	434 ml	
FBS	50 ml	10 %
Penicillin/Streptomycin	5 ml	1 %
GlutaMAX supplement (100x)	5 ml	1 x
HEPES (1 M)	5 ml	10 mM
Primocin (50 mg/ml)	1 ml	100 µg/ml

Growth media (50 ml)

Media and Growth factor	Addition amount	final concentration
Basal media	48.8 ml	
FGF7 (10 µg/ml)	120 µl	24 ng/ml
BMP4 (50 µg/ml)	50 µl	50 ng/ml
Activin A (100 µg/ml)	50 µl	100 ng/ml
n-Acetyl cysteine (100 mM)	500 µl	1 mM
[Leu15]-Gastrin I (10 mM)	50 µl	10 nM
EGF (0.1 mg/ml)	25 µl	50 ng/ml
FGF10 (0.1 mg/ml)	50 µl	100 ng/ml
A83-01 (50 mM)	1 µl	1 µM
DAPT (50 mM)	10 µl	10 µM
Dexamethasone (500 mM)	1 µl	10 µM
R-spondin1 (0.1 mg/ml)	250 µl	500 ng/ml
HGF (0.1 mg/ml)	25 µl	50 ng/ml
VEGF (50 µg/ml)	25 µl	25 ng/ml
bFGF (0.1 mg/ml)	25 µl	50 ng/ml

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2-3. Preparation of working medium

Add Penicillin/Streptomycin (PS) to DMEM/F12 and prepare 50 ml of DMEM/F12 containing final 1 % PS (used as working media (serum -). Prepare another 50 ml of working media (serum +) containing 10 % final fetal bovine serum and 1 % PS, and store on ice.

3. Isolation of liver from mouse embryo

3-1. Prepare all reagents on ice. Prepare dissection tools, dishes, mouse, and stereo microscope in a laminar flow cabinet.

3-2. Open the abdomen of a female mouse (12th day of pregnancy) in a laminar flow cabinet and remove the embryo in uterus. Place the removed uterus (with embryo) into a 6 cm dish containing 3 ml of working media (serum -). Transfer the embryo into a 6 cm dish containing 3 ml fresh working media (serum -) for washing.

3-3. Remove amniotic membrane and the placenta form the embryo.

3-4. Transfer the embryo to a 6 cm dish containing 5 ml of working media (serum -) and store on ice.

3-5. Place the embryo into the lid of a 6 cm dish containing 2 ml of working media (serum -), obtaining organs on a laminar flow cabinet under a stereomicroscope.

3-6. Under a stereomicroscope, dissect the embryo using two 1 ml syringes fitted with 27 G needles, and obtain the developing liver organs (rough dissection is done with an 18 G needle, and detailed dissection is done with a 27 G needle).

3-7. Transfer extracted organs to a 6 cm dish containing working media (serum -) using a pipette and store on ice.

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4. Isolation of cells from embryonic liver and dispersion into single cells

4-1. Place extracted embryonic liver organs into a 6 cm dish containing 5 ml of Accutase. Mince the tissue using scissors. After pipetting well, incubate at 37 °C for 10 minutes.

4-2. Stop the enzyme treatment by adding 2 ml of working media (serum +) and pipetting well .

4-3. Equip a 40 µm filter in a 50 ml conical tube.

4-4. Using a pipette, place the Accutase-treated tissue on the filter.

4-5. Filter the tissue using cell scraper.

4-6. Wash the filter with working media (serum +) (1 ml x 3).

4-7. Collect the solution stuck on the back of the filter with a pipette. Combine the cell suspension collected under the filter process 4-5 and 4-6 and transfer to a 15 ml tube.

4-8. Store cells in 15 ml tube on ice. Dilute aliquot (10 µl) 10 times and count the number of cells.

4-9. Prepare 15 ml tubes for the number of embedding culture experiments. Dispense the cell suspension into each 15 ml tube (2.5~5 x 10^5 cells in 200 µl gel culture is recommended). Centrifuge at 200 x g for 5 minutes to reaggregate the cells.

4-10. Discard the supernatant with a pipette and store the cell pellet on ice.

5. Preparation of 3D culture substrate for embedding

Making MatriMix (511) complete solution: Mix MatriMix (511) kit regent (A+B+C) according to the protocol in the instruction manual and store on ice. Amount of Preparing MatriMix (511) complete solution is total 200 µl for 1 experiment on one well in a 12-well cell culture plate. * Using 6 or 12-well cell culture plate, total 200 µl gel is recommended and using 24-well cell culture plate, total 100 µl gel is recommended (Change the amount of gel depending on user method).

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6. MatriMix (511) gel-embedded culture of embryonic liver cells

6-1. Add 200 µl of MatriMix (511) complete solution to prepared cell pellet (4-10) and mix well with a pipette (be careful not to introduce air bubbles).

6-2. Using pipette, drop cell mixture with MatriMix (511) in a dorm shape onto a cell culture plate (glass bottom dish can also be used instead), and leave in a CO2 incubator at 37 ˚C for 30 minutes.

6-3. Visually check that MatriMix has turned into a gel, then gently drop the 37 ˚C pre-warmed growth media from the side of the gel.

6-4. Culture in a CO2 incubator at 37 ˚C.

After seeding, culture in growth media for 2 days. After 2 days culture, change the media to basal media. Thereafter, replace the media with basal media every 3 days and culture for 7 days (medium composition is described in chapter 2).

References

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