Midbrain Neuron-glia Mixed Cultures

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[Abstract] Mixed neuron-glia cultures provide a unique tool to study cellular contribution and molecular pathways in various neurological disorders. They are also invaluable for exploring neuron-glia interaction under physiological and pathological conditions. The relatively long-lasting midbrain neuron-glia mixed cultures generated following this protocol have been widely used to study the pathogenesis of Parkinson's disease, the most common neurodegenerative movement disorder.

Materials and Reagents

1. Poly-D-lysine (Sigma-Aldrich, catalog number: P7280)
2. MEM (Life Technologies, Gibco®, catalog number: 11090-08)
3. D-Glucose
4. Sterile water
5. Sterile PBS
6. Trypan blue dye
7. Heat-inactivated fetal bovine serum (FBS) (Life Technologies, Gibco®, catalog number: 16000-044)
8. Heat-inactivated horse serum (HS) (Life Technologies, Gibco®, catalog number: 26050-088)
9. None essential nonessential amino acids (Life Technologies, Gibco®, catalog number: 11140-050) (100 ml)
10. Sodium pyruvate (Sigma-Aldrich, catalog number: S8636) (100 ml)
11. 200 mM L-glutamine (Life Technologies, Gibco®, catalog number: 25030-081) (100 ml)
12. Penicillin/streptomycin (Sigma-Aldrich, catalog number: P0781) (100 ml)
13. Poly-D-lysine stock solution (see Recipes)
14. Maintenance culture medium (see Recipes)
15. Treatment medium (see Recipes)

Equipment

1. Cell culture incubator
2. Standard benchtop centrifuges
3. Hemocytometer
4. Dissection microscope
5. Scissors and forceps
6. Sterile filter (0.2 µm)
7. Foil
8. 24-well plates
9. Laminar hood
10. 50-ml tube
11. 10-ml pipet

Procedure

1. Coating and washing culture plates
   a. In a laminar hood, dilute poly-D-lysine stock solution (5x) with sterile water to 20 µg /ml.
   b. Add 0.25 ml to each well of 24-well plates.
   c. Leave the plates in the hood for 2-3 h or in the in incubator for at least 1 h.
   d. Before use, remove the coating solution.
   e. Wash the wells twice with 1 ml/well of sterile water.
   f. Add 1 ml sterile PBS to each well. Completely remove the PBS right before use.
2. In the animal procedure room, remove embryos from time-pregnant rats or mice at embryonic day 13/14 and place embryos in cold MEM.
3. Under a microscope, dissect out the midbrain portion of the embryonic rat or mouse brains. Remove meninges and blood vessels. Pool tissues and keep in ice cold MEM.
4. In a laminar hood, transfer tissues to a 50-ml tube. Gently triturate the tissues (5-10 times each) first with a 10-ml pipet, then with a 1-ml pipet tip fitted to the 10-ml pipet followed by a fitted 200 µl pipet tip.
5. Centrifuge the triturated tissues for 10 min at 6.5x speed setting (~1,500 rpm).
6. Carefully remove the supernatant and resuspend the pelleted cells in 10 ml of maintenance culture medium.
7. Take 30 µl of the cell suspension and mix with 270 µl of Trypan blue dye. Load 10 µl onto a hemocytometer to count cell density.
8. Adjust the cell density to 1 x 10^6 cells/ml with maintenance culture medium.

9. Add 0.5 ml of cells to each well of the poly-D-lysine-coated 24-well plate.
10. Place the plates in a humidified 37 °C incubator with 5% CO_2.
11. Three days after the initial seeding, add 0.5 ml of warm (37 °C) maintenance culture medium to each well.
12. Seven days after initial seeding, cultures will be ready for treatment with vehicle or desirable reagents in treatment medium.
13. At the time of treatment, the neuron-glia cultures are made up of ~10% microglia, 50% astrocytes, and 40% neurons of which 2-3% are tyrosine hydroxylase-immunoreactive neurons.

Recipes

1. Poly-D-lysine solution
   Dissolve in 50 ml of ddH_2O to make 5x stock solution.
   Keep as 5.0 ml aliquots at -20 °C.
   Dilute with sterile ddH_2O right before use.
2. Maintenance culture medium

Reagents	**volume**	**final con.**
MEM	380 ml	-
D-Glucose	0.5 g	1 g/L
Heat-inactivated fetal bovine serum *	50 ml	10%
Heat-inactivated horse serum **	50 ml	10%
None essential nonessential amino acids	5 ml	0.1 mM
Sodium pyruvate	5 ml	1 mM
L-glutamine	5 ml	2 mM
Penicillin/streptomycin	5 ml	50 U/ml/50 µg/ml

   Con.: concentration
   Sterile filter (0.2 µm) and store wrapped in foil at 4 °C.
   *Heat-inactivated at 56 °C for 30 min and stored in 50 ml aliquots at -70 °C.
   ** Stored in 50 ml aliquots at -20 °C.
3. Treatment medium

Reagents	**volume**	**final con.**
MEM	465 ml	-
Heat-inactivated FBS	10 ml	2%
Heat-inactivated HS	10 ml	2%
Sodium pyruvate	5 ml	1 mM
L-glutamine	5 ml	2 mM

Penicillin/streptomycin	5 ml	50 U/ml/50 µg/ml

Sterile filter (0.2 µm) and store wrapped in foil at 4 °C.

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References

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