Generation of Microglia Cultures and Mixed Glial Culture

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[Abstract] Primary rodent microglia-enriched cultures are the most popular model to study microglial biology *in vitro* and to explore immune signaling pathways. Mixed glial cultures that contain microglia and astroglia are very useful for investigating the precise mechanisms of microglia-astroglia interaction during immune reaction. This protocol has been developed and improved over the years by various researchers in Dr. Hong's lab, especially Dr. Bin Liu.

Materials and Reagents

1. Poly-D-lysine (Sigma-Aldrich, catalog number: P7280)
2. DMEM/F12 (Life Technologies, Invitrogen™, catalog number: 11330-032)
3. D-Glucose
4. Sterile water
5. Sterile PBS
6. Heat-inactivated fetal bovine serum (FBS) (Life Technologies, Gibco®, catalog number: 16000-044)
7. Non-essential amino acids (100 ml) (Life Technologies, Gibco®, catalog number: 11140-050)
8. Sodium pyruvate (100 ml) (Sigma-Aldrich, catalog number: S8636)
9. 200 mM L-glutamine (100 ml) (Life Technologies, Gibco®, catalog number: 25030-081)
10. Penicillin/streptomycin (100 ml) (Sigma-Aldrich, catalog number: P0781)
11. DMEM/F12-based culture medium (see Recipes)
12. Poly-D-lysine stock solution (see Recipes)
13. Treatment medium (see Recipes)

Equipment

1. Cell culture incubator
2. Centrifuges
3. Dissection microscope
4. Scissors and forceps

5. T175 flasks
6. 70 µm nylon filter (sterile)
7. 50 ml tube
8. Sterile filter (0.2 µm)
9. Foil
10. Laminar hood

Procedure

1. Seed cells from 2-2.5 rat brains or 4-5 mouse brains from pups of postnatal day 1 to 5 in to one T175 flask.
2. Coat T175 flasks with 20 ml Poly-D-lysine (20 µg ml^{-1}) in a laminar hood for 2-3 h or in the in incubator for at least 1 h.
3. Wash the flasks twice with 25 ml of sterile water. Add 25 ml sterile PBS to each flask.
4. In the animal procedure room, remove whole brains from 1-3 day-old rat or mouse pups and place them in cold DMEM/F12.
5. Under a microscope, remove olfactory bulb and cerebellum from the brain. Remove meninges and blood vessels.
6. Pool tissues from up to 10 brains into a 6 cm petri dish and keep dish on ice.
7. In a laminar hood, transfer tissues to a 50-ml tube. Gently triturate the tissues (5-10 times each) first with 10 ml pipet, then a 1 ml pipet tip fitted to the 10 ml pipet followed with a fitted 200 µl pipet tip.
8. Filter the minced tissue suspension through a 70 µm nylon filter and centrifuge the filtrate for 10 min at 6.5x speed setting (~1,500 rpm).
9. Carefully remove the supernatant using 10 ml pipet and resuspend the pelleted cells in 10-20 ml of DMEM/F12-based culture medium.
10. Completely remove the PBS from flasks. Add 25 ml of pre-warmed (37 °C) DMEM/F12-based culture medium to each flask.
11. Resuspend the cell suspension again and put the cells to each flask preloaded with medium. Place the flasks into a humidified 37 °C incubator with 5% CO_2.
12. Four days after the initial seeding, remove the medium and add 25 ml of fresh warm culture medium to each flask.
13. Mixed glia cultures will be ready for shaking off microglia at between 12-14 days after initial seeding.
14. Separate microglia from astrocytes by shaking the flasks for 5 h at 180 rpm. The enriched microglia are >98% pure as determined by OX-42-immunostaining and glial fibrillary acidic protein (GFAP)-immunostaining.

Recipes

1. Poly-D-lysine solution
   Dissolve in 50 ml of ddH_2O to make 5x stock solution.
   Dilute with sterile ddH_2O to 20 µg/ml right before use.
2. DMEM/F12-based culture medium

Reagents	volume	final con.
DMEM/F12	430 ml	-
Heat-inactivated fetal bovine 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

   on.: 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.
3. Treatment medium

Reagents	volume	final con.
DMEM/F12	470 ml	-
Heat-inactivated fetal bovine serum*	10 ml	2%
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

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

References

1. Gao, H. M., Hong, J. S., Zhang, W. and Liu, B. (2002). Distinct role for microglia in rotenone-induced degeneration of dopaminergic neurons. *J Neurosci* 22(3): 782-790.
2. Liu, B., Du, L. and Hong, J. S. (2000). Naloxone protects rat dopaminergic neurons against inflammatory damage through inhibition of microglia activation and superoxide generation. *J Pharmacol Exp Ther* 293(2): 607-617.

