Preparation of Astroglia Cell Culture

References

1. Ferreira, A., Sinjoanu, R. C., Nicholson, A., & Kleinschmidt, S. (2011). Aβ toxicity in primary cultured neurons. *Methods in molecular biology (Clifton, N.J.)*, *670*, 141–153. https://doi.org/10.1007/978-1-60761-744-0_11
2. Ferreira, A and Loomis, P.A. Isolation and culture of primary neural cells. In: "Cells: A laboratory Manual" Cold Spring Harbor Laboratory. Ed. By D. Spector, R. Goldman and L. Leinwand. Pp 9.1-9.13, 1998.
3. Banker, G and Goslin, K. (1998). Rat hippocampal neurons in low-density culture. In: Culturing nerve cells (Banker G, Goslin K, eds), pp339-370. Cambridge: MIT PS.

Solutions

1. Calcium and magnesium-free Hank's balanced salt solution (CMF-HBSS): 1X HBSS (Invitrogen), 9.9 mM HEPES and 1 U/100 μg of penicillin/streptomycin in water. Store solution at 4°C.
2. Glial and neuronal plating medium (MEM 10): Minimum essential medium (1X MEM, Invitrogen), 10% heat inactivated horse serum (Invitrogen), 0.6% D-glucose, 1 U/100 μg penicillin/streptomycin. Store solution at 4°C.
3. Neuronal maintenance medium (N2): 1X MEM (Invitrogen), 0.6% D-glucose, 0.1% ovalbumin, 1 mM sodium pyruvate, 5 μg/ml insulin, 20 nM progesterone, 100 μM putrescine, 30 nM selenium dioxide, 100 μg/ml apo-transferrin. Filter-sterilize and store at 4°C. Keep component stock solutions at -20°C.

Abbreviations

1. E18: Embryonic day 18
2. CMF-HBSS: Calcium and magnesium-free Hank's balanced salt solution
3. MEM 10: Glial and neuronal plating medium
4. N2: Neuronal maintenance medium

Protocol

Dissection

1. Sacrifice a pregnant Sprague-Dawley rat, embryonic day 18 (E18; see Notes 1 and 2) using an approved method of euthanasia.
2. Saturate the abdomen of the rat with 75% ethanol before making an incision.
3. Using sterile forceps and scissors, remove the skin to expose the muscular abdominal wall by making a vertical incision from pelvis to thorax.
4. Soak the used instruments with ethanol to remove any hair (see Note 3).
5. Cutting through the abdominal wall, expose the uterus.
6. Remove the uterus of the rat and place it in a sterile 100 mm dish (see Note 4). Continue to work in a laminar flow hood.

7. Remove the fetuses from the uteri using sterile scissors and forceps (Dumont #3).
8. Decapitate the fetuses and transfer the heads to a 60 mm dish containing 3-4 ml CMF-HBSS.
9. To remove the brains, place a Dumont #3 forceps into the orbits to stabilize the head. Using a Dumont #5 forceps, peel away the skin and the skull to expose the brain.
10. Remove the brains and place them onto a drop of CMF-HBSS in the center of a 35 mm sterile dish coated with sterile paraplast (see Note 5).
11. Remove the meninges from each hemisphere (see Note 6).
12. After the dissection of the hippocampus (see Neuronal Culture Dissection Step 1.1), cut a quarter of each hemisphere's cortex. Use approximately 6-8 hemispheres (24-36 quarters).
13. Transfer the cortical sections to a sterile 60 mm dish containing 3-4 ml of CMF-HBSS.

Dissociation and Plating

1. Aspirate the tissue using a sterile transfer pipette and transfer it to a 15 ml sterile conical tube that contains 15 ml 0.25% trypsin solution in CMF-HBSS. Invert the tube to mix.
2. Incubate in a water bath at 37°C for 30-35 min for digestion.
3. Using a sterile transfer pipette, aspirate the tissue from the 15 ml conical tube and transfer it to a 50 ml sterile conical tube.
4. Pipette up and down 10-12 times with a Pasteur pipette to triturate the tissue.
5. In the same tube, add 30 ml of glial plating medium (MEM 10) to inactivate the trypsin.
6. Spin this mixture for 10 min at 800 x g to pellet the cells.
7. Discard the supernatant and add 20 ml of fresh MEM 10 to the pellet.
8. Using a Pasteur pipette, dissociate the tissue 10-15 times. Then use a fire-polished Pasteur pipette (the tip diameter of which should be half of the regular diameter) and dissociate until the solution becomes homogenous.
9. Increase the total volume of MEM 10 to 50 ml before plating.
10. Determine the cell density using a hemocytometer and dilute the cell suspension to approximately 200,000 cells/ml and plate them into 60 mm dishes (see Notes 7 and 8).
11. Complete the volume in each dish up to 4 ml using MEM 10.
12. Swirl the dishes to spread cells evenly.
13. Incubate cultures at 37°C with 5% CO_2.

Maintenance

Four days later, remove all media and dead cells from the dishes and add 4 ml fresh MEM 10. The glial cultures should be fed once a week with fresh MEM 10 (see Notes 9 and 10).

NOTES

1. We use E18 rat embryos because at this stage, the neuronal migration is complete and the hippocampus is formed. For mouse neuronal hippocampal cultures, we follow the same protocol, except the age of the embryos is 16 days.
2. For glial culture, we use the cortex from the E18 rats to be used for hippocampal culture preparations, to minimize the number of animals sacrificed for each culture. The same culture can be done using postnatal rat pups (1 or 2 days old; ref. 2).

3. Hair and skin are a source of fungal and bacterial contamination.
4. When removing the uterus, be cautious as not to pinch the nearby intestine, which is a source of bacterial contamination.
5. The 35 mm dishes used for dissection of the hippocampi are coated with sterile paraplast to better stabilize the brain hemispheres and to protect the delicate tips of our surgical instruments. We coat each dish with the same volume of paraplast in order to minimize changes in the focal plane of the dissecting microscope.
6. Meninges are a source of contamination with different cell types, which secrete toxins that do not support neuronal survival.
7. We plate the cells at two different densities, so as to have cultures ready at different times.
8. We plate and grow the glial culture in sterile 60 mm dishes for easier use in co-culturing with neurons. Glial cell suspension can be plated and expanded in T-flasks, harvested and then re-plated.
9. Use the glial cultures for conditioning of the N2 media once the cultures reach 40-60% confluence, usually in 10-14 days.
10. For the long-term survival of neurons, we use N2 media that has been glia-conditioned for 24 h.