Protocol for preparing primary sandwich hippocampal neuron cultures for cryo-electron tomography

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Abstract

Primary sandwich hippocampal neuron cultures are adapted from the Kaech and Banker protocol (Kaech and Banker, 2006) and provide neuronal cultures with almost no glial cells, which may facilitate the targeting of neurons for cryo-electron tomography (see accompanying protocol by Siegert, Petrovic, Do et al.). In addition, the cells can be seeded at a very low density.

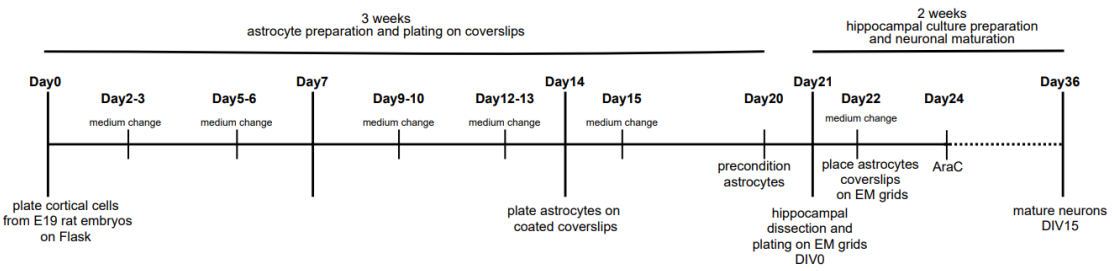

Fig.1: Timeline of primary sandwich hippocampal neuron culture preparation. Astrocytes are cultured for 3 weeks prior to plating on coverslips. Following the hippocampal dissection, primary hippocampal cultures are plated on EM grids, and neuronal maturation takes place in the presence of the astrocyte coverslips and arabinofuranoside.

1. Preparation of the EM grids

For preparation of EM grids, follow the accompanying protocol by Siegert, Petrovic, Do et al.

Note: Only 35 mm dishes with 4 inner rings are suitable for the preparation of primary sandwich hippocampal neuron cultures.

2. Preparation of primary sandwich hippocampal neuron culture on EM grids

Astrocyte feeder layer

Materials: T75 flasks; 10 mL serological pipettes; 15 mL sterile tubes; 25 mm sterile coverslips; MEM 1X (Gibco) supplemented with 2 mM L-Glutamine, 10 % Horse Serum (Gibco) and 4.65 g/L of glucose (MEM10%HS); 0.1 mg/mL poly-L-lysine; sterile water; 0.05 % Trypsin/0.02 % EDTA; DMSO; centrifuge for 15 mL tubes.

1. Plate 5 million cortical cells from an E19 rat embryo cortical suspension in T75 flask in MEM10%HS.

2. Change the medium of the astrocytes twice a week with fresh MEM10%HS pre-equilibrated at 37 °C and 5 % CO_2. Slam the flask against the bench surface before aspirating the medium to remove the microglial cells. Two weeks after the dissection, the astrocyte cultures should be more than 50 % confluent.

Note: The choice of a specific medium as well as the removal of microglia (as described in the previous step) favours the proliferation of astrocytes over neurons and other glia in the cortical cell suspension.

3. 6-7 days before the hippocampal dissection (2 weeks after the cortical dissection; Fig. 1), coat 25 mm sterile coverslips with 0.1 mg/mL poly-L-lysine for 15 min at RT. Wash the coverslips 3 times with sterile water and replace the water with MEM10%HS.

4. Harvest the astrocytes from the flask: Slam the flask and aspirate the medium. Quickly wash the flask with 0.05 % Trypsin/0.02 % EDTA. Add 2 mL of trypsin/EDTA and incubate 2 min at 37 °C until the cells detach. Stop the trypsination by adding 5 mL of MEM10%HS. Release the cells by multiple rounds of pipetting with a 10 mL serological pipette. Transfer the cells to a 15 mL sterile tube and centrifuge for 5 min at 500 g. Re-suspend the cells in 2 mL of MEM10%HS complete medium. Count the cells and plate them dropwise on each of the coverslips: use 200,000 cells in a total volume of 100 µL of medium per coverslip.

Note: The remaining astrocytes in MEM10%HS complete medium with 10 % DMSO can be frozen and kept in liquid nitrogen storage for more than a year. They can be thawed six days before the hippocampal dissection.

5. One day after plating, change the medium of the astrocytes again with fresh pre-equilibrated MEM10%HS.

Primary neuron cultures with astrocyte feeder layer

Materials: sterile tweezers; Neurobasal medium supplemented with 2 % B27 and 2 mM L-Glutamine; DMEM10%FCS; arabinofuranoside (cytosine-β-arabinofuranoside hydrochloride)

1. One day before the hippocampal dissection, precondition the astrocyte feeder layer in Neurobasal medium + 2 % B27 + 2 mM L-Glutamine pre-incubated at 37 °C and 5 % CO_2 for few hours.

2. Dilute the hippocampal suspension to reach a density of 200,000 to 350,000 cells per mL in DMEM10%FCS.

3. Immediately after removing the water from the compartments of the dishes plate 100 µL of cell suspension dropwise on each EM grid (Fig. 2, left). Incubate at 37 °C and 5 % CO_2.

4. After two hours, add 500 µL of pre-heated DMEM10%FCS per dish.

5. Incubate overnight at 37 °C and 5 % CO_2.

6. Replace the plating medium with 2 mL of pre-conditioned Neurobasal medium from the astrocyte feeder layers' dish.

7. With sterile tweezers take the coverslip with the astrocyte layer and flip the coverslip so that the neurons are facing the astrocytes (Fig. 2, right).

8. To stop the proliferation of glial cells on the grids, treat the primary neuron cultures with the anti-mitotic agent arabinofuranoside (cytosine-β-arabinofuranoside hydrochloride) three days after plating the hippocampal cells. Add arabinofuranoside to a final concentration of 2.45 µM to each dish and distribute evenly by gently moving the dish in a circular motion.

9. The neurons are considered mature starting from 15 days *in vitro* (DIV15).

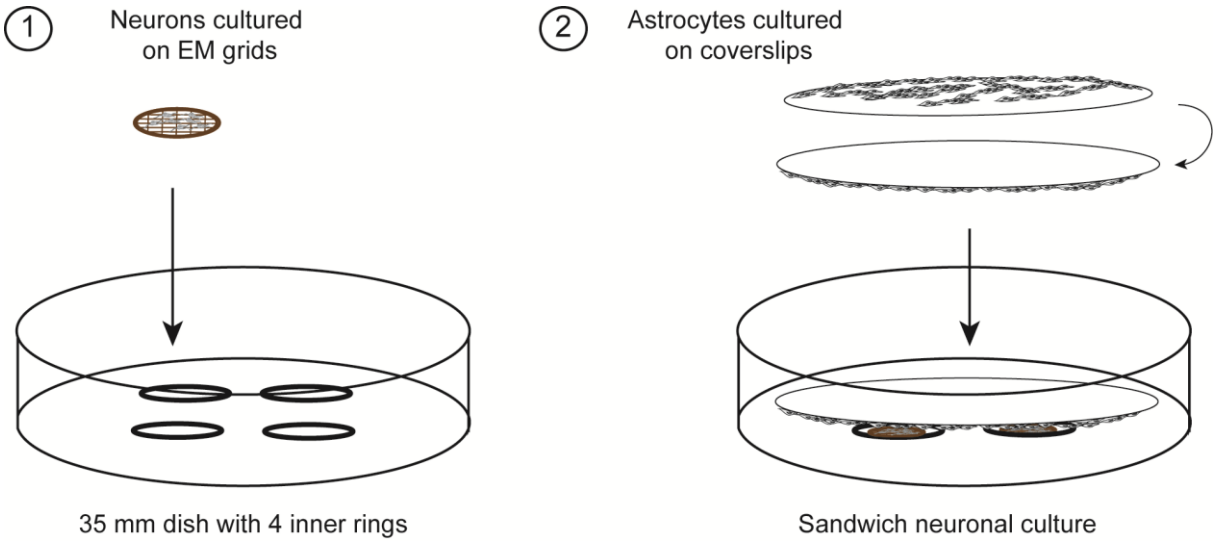

Fig 2: Schema of the sandwich cultures on EM grids

3. Reference

Kaech, S., and Banker, G. (2006). Culturing hippocampal neurons. Nature Protocols *1*, 2406-2415.