III-6 [Culture of Rat Oligodendrocytes]

6-1. Materials

6-1-a. Animals:
- Pregnant Wistar rats: embryonic day 16 (E16) for OPC and OL.

6-1-b. Special equipment:
- Sterile microdissection kit (two pairs of fine-tipped forceps and small surgical scissors, micro spring scissors) (Roboz, USA).
- Dissecting microscope (Nikon, Japan).
- Refrigerated centrifuge (Kokusan, H-3R, Japan).
- Water-jacketed humidified CO$_2$ incubator (Napco, Japan).
- Sterile 15 and 50 ml centrifuge tubes (Corning, USA).
- Pasteur pipettes (Corning, USA).
- Sterile 5 and 10 ml plastic pipettes (Falcon, USA).
- Culture dishes (Griner, Germany).
- Petri dishes (6 and 10 cm diameters) (Sterilin, UK).
- Circular coverslips 13 mm diameter for cell culture (Matumani, Japan).
- 140 µm pore-sized stainless mesh (JIS, Japan).
- Cell strainer (40 µm nylon) (Falcon, USA).
- 10 µm pore-sized nylon mesh (NITEX, Switzerland).
- Fluorescence microscopy with Olympus confocal laser scanning FV1000 system with an inverted microscope (Olympus, Japan).

6-2. OPC and OL culture:
Step 1. OPC and OL cultures were prepared from an E16 rat cerebrum by the modification of the method of previously described methods. All fetuses from a pregnant rat were pooled for a culture preparation.

Step 2. Fetal rat brains were removed by dissection of the skull using sterilized fine-tipped forceps and micro spring scissors in sterile ice-cold HBSS. Cerebral cortices were dissected from the whole brain and meninges and blood vessels were carefully removed.

Step 3. The rat embryonic cerebral cortex from was mechanically dissociated through 140 µm pore-sized stainless mesh in 10% FCS in E-MEM.
Step 4. The cells were dissociated with a fire-polished Pasture pipette. Trituration involved pipetting the tissue suspension in and out of the pipette approximately 20 times. A 15 ml tube of cells was left still for 5 min to allow the tissue debris to settle down. The supernatant was transferred to a fresh 15 ml tube, and then was centrifuged for 10 min at 1000 rpm at 4 °C. Cells were resuspended in 10% FCS/E-MEM and finally sieved through 40 µm pore-sized nylon mesh. This step was repeated twice. The number of viable cells was determined by trypan blue exclusion in a hemocytometer.

Step 5. Dispersed cells were seeded on PLL (100 µg/ml)-coated 90 mm diameter culture dishes at a density of 1x10^7 cells/dish. Cells were incubated in an atmosphere of 90% air, and 10% CO₂ and approximately 98% humidity at 37 °C.

Step 6. After 6 days culture, the cells were passaged with 0.05% trypsin in D-PBS (1st passage). Cells were resuspended in 10% FCS/E-MEM and were finally sieved through 10 µm pore-sized nylon mesh were cultured for 7 days at a density of 8x10^6 cells per none-coated culture dish.

Step 7. After 6 days culture, cells were passaged with 0.05% trypsin in D-PBS and were cultured for 2 days in 10% FCS/E-MEM at a density of 3x10^6 cells per none-coated Petri dish (2nd passage).

Step 8. On the 2nd day of culture, the medium was exchanged to serum-free chemical defined D-MEM (CDM) supplemented with 10 µg/ml insulin, 0.5 µg/ml transferrin, 100 µg/ml BSA, 60 ng/ml progesterone, 16 µg/ml putrescine, 40 ng/ml sodium selenite, 60 ng/ml N-acetyl-L-cysteine and 10 ng/ml PDGF-AA, and the OPC were cultured for another 2 days.

Step 9. To differentiate OPC, the cells were continuously cultured with 30 ng/ml T₃, 40 ng/ml T₄, 10 ng/ml NT-3 and without PDGF for 3~5 days. These procedures are necessary to eliminate neurons and astrocytes.