Chapter 12

Embryonic Rodent Brain Cells in Culture

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1. Introduction

Because of its cellular complexity and regional heterogeneity, the mammalian central nervous system is not easily amenable for experimental analysis. The study of the developing brain becomes even more complicated because of the differential growth rates of different parts of the brain. Primary culture techniques involving dissociation of discrete regions of the developing brain into component cells offer an excellent opportunity to study the regulation of growth and differentiation of neural cells and to investigate their biochemical, morphological, and physiological behavior under well-defined conditions. Methods are available now to isolate and grow individual classes of neural cells (i.e., neurons, astrocytes, oligodendrocytes), thereby enabling one to study the cellular behavior at the individual level and to uncover the nature of cell–cell interactions that presumably govern cell differentiation.

The procedure widely used to prepare primary cultures of a mixed neuronal-glial population involves dissociation of embryonic rat/mouse brain. The cultures can be maintained either as suspension cultures or as surface cultures. Both systems recapitulate normal developmental events, and thus, have proved highly useful for the study of neuronal and glial differentiation (*1*,*2*). Described here is a version of the method to set up surface-adhering primary cultures of embryonic rodent brain cells.

2. Materials

1. Instruments: Surgical instruments such as microforceps (straight and curved; sharp and blunt-pointed), dissecting scissors, nylon mesh (20 and 78 μm sieve size), glass pestle (Belco).
2. Animals: Pregnant (15 d) rats/mice.
3. Dulbecco's Modified Eagle's Medium (DMEM): To prepare DMEM, dissolve the powdered medium, sodium bicarbonate (3.7 g/L), glucose (5 g/L) and antibiotic-antimycotic mixture (10 mL/L) representing 100 U/mL penicillin, 100 μg/mL streptomycin, and 250 ng/mL amphotericin B in an appropriate vol of distilled, deionized water. After adjusting its pH to 7.4, the medium is filter-sterilized.

3. Method

Before the start of the experiment, make sure all the instruments, nylon mesh, paper towels, and small pieces (~1 in^2) of Whatman No. 1 paper are sterilized. Warm up the medium to room temperature.

Turn on the laminar flow hood and light the burner. Place the sterile surgical instruments in a beaker containing 70% alcohol. Also, keep 100 x 20-mm dishes containing DMEM ready.

1. Removal of the embryos: Outside the hood, sacrifice the pregnant rats by CO_2-gassing. Wipe the abdominal area with 70% alcohol. Remove the embryos by Caesarean section and place them in a culture dish containing DMEM. The rest of the procedure is to be carried out under the hood.
2. Dissection: Force out the fetuses from the sacs with a gentle cut with the scissors. Individual embryos are wiped off by gently rolling on a sterile paper towel. Dissect the head and place it upright in an empty dish. Then, holding the front end down with a forceps, remove the entire brain with another forceps through a midline incision in the skull made with scissors. The brains thus taken out are collected in a dish containing DMEM. The cerebral hemispheres may then be separated from the rest of the brain with a cut across the mid brain.
3. Removal of meninges: This is done under a dissecting microscope kept in the hood. Place the cerebral tissue on a piece of sterile paper (Whatman No. 1) kept in a dish (60 x 15 mm) containing 4 mL DMEM. Looking through the microscope, one can visualize the blood vessels embedded in the meningeal membrane covering the tissue. Remove the meningeal membrane by probing through it with pointed forceps.

If done correctly, the entire membrane should peel off in one piece.

4. Dissociation: Cerebra are then dissociated mechanically. The tissue is first smoothed in a culture dish with the aid of a glass pestle, then suspended in medium containing 15% FCS, and dispersed by passing the suspension through a sterile pipet several times. Further dissociation is carried out by sequential passages through nylon meshes of pore sizes 78 μm and 20 μm. The resulting suspension of dissociated cells is centrifuged at a low speed (150 x g, 5 min) to obtain the pellet. The pelleted cells are washed twice with the medium and suspended at a density representing 2.5 x 10^6 cells/mL. Final plating density: 3 x 10^5/cm^2 surface area.
5. Maintenance of cultures: The initial change of medium is after 3–4 d of culture (when most cells have attached to the surface of the dish/flask) and twice a week thereafter. DMEM containing 10% FCS is used for maintaining the cultures.

4. Notes

1. The dissociated embryonic brain cells have a tendency to reaggregate. The aggregates of cells thus formed attach to the surface of the dish and grow fibrous processes. By about a week in culture, an extensive network of neuritic processes can be seen covering the surface of a layer of proliferating astroglial precursors (Fig. 1). Cell proliferation, mostly accounted for by glial cells, peaks around 6 d in culture (3). This proliferative period is followed by cell differentiation, as indicated by an increase in neuronal and glial properties (e.g., *4–7*). The time course of cell differentiation corresponds closely to the in vivo pattern, and thus, the culture system serves as an excellent model of the developing brain.
2. It should be pointed out that, under the culture conditions described here, neurons start degenerating after the second week. Thus, if the experiments call for prolonged neuronal survival, the following modifications may be incorporated.
   a. Use of antimitotic agents: An overgrowth of nonneuronal (glial) cells in these cultures seems to be the main reason for neuronal decay. In order to prevent this, investigators have used antimitotic agents such as fluorodeoxyuridine and cytosine arabinoside (*8*). Thus, for example, the cultures are set up as described (the seeding cell density may be reduced to one-half). The cultures grown for 6–8 d are treated with the inhibitor at a concentration of 1 x 10^{-5}*M* for 24 h.

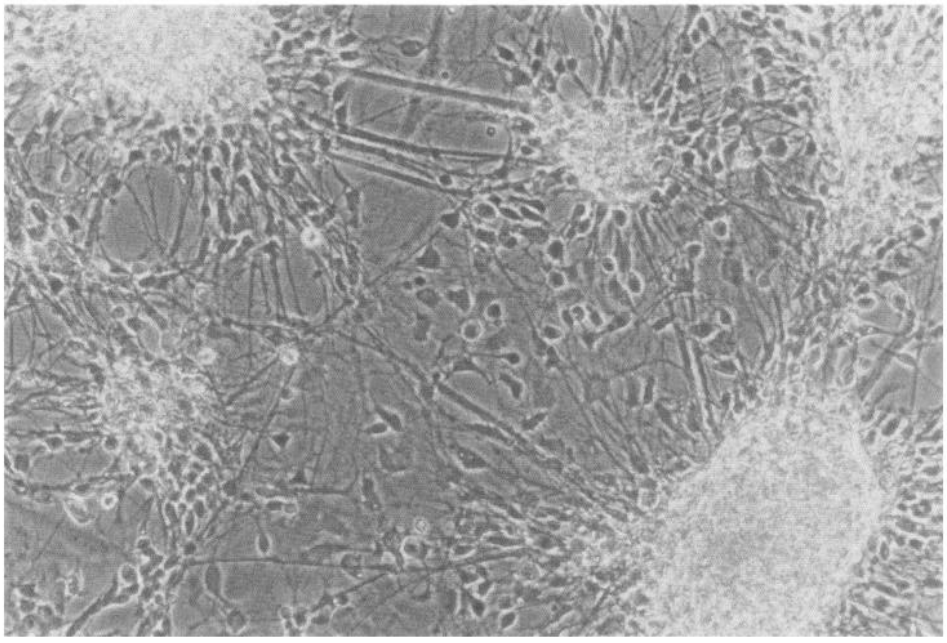

Fig. 1. Phase contrast micrograph of a 7-d-old primary culture established from 15-d embryonic rat cerebral tissue.

b. It has been noticed that supplementation of the growth medium with a combination of fetal calf serum and horse serum results in a reduced glial growth, thereby promoting neuronal survival. Better still, Honegger et al. (*9*) have described a serum free defined medium [based on the N-2 medium originally described by Bottenstein and Sato (*10*)] that seems to support the long-term growth of mixed neuronal-glial cell populations. A similar growth medium has been used to study neuronal differentiation in surface-adhering cultures of fetal mouse hypothalamic cells (*11*).

3. Pettman et al. (*12*) have described a technique to grow pure neuronal cultures from embryonic chick brain. In this procedure, dissociated cells from 8-d embryonic brain are seeded onto polylysine-coated culture dishes. Glial cells fail to proliferate under these conditions for two reasons: (a) the early embryonic age chosen when gliogenesis is minimal and (b) the growth of few glial cells present in the initial cell suspension is inhibited by the polylysine substrate.

Alternatively, the procedure described by Hanson et al. (*13*) separates neurons and glial cells based on (a) the differential adhesion properties of neurons and nonneuronal cells to a collagen substrate and (b) the capacity

of neurons to form homotypic aggregates. Briefly, culture flasks are coated with specially treated rat tail collagen. The cell suspension is plated on collagen-coated flasks and placed in an incubator on an intermittantly agitating platform. The nonneuronal cells progressively attach to the substrate while the neurons form homotypic neuronal aggregates in the supernatant. These aggregates are then triturated with a Pasteur pipet and replated on a fresh flask coated with collagen. The original flasks are rinsed free of the remaining neurons with fresh medium to obtain glial enriched cultures.

The above procedures originally used to prepare culture of embryonic chick brain cells can easily be adapted for embryonic rat/mouse brain cells. It is a fact that the cellular composition of primary brain cell cultures varies with the age of the donor tissue. Thus, cultures established from embryonic cortex generally results in a mixed neuronal-glial population, whereas cultures prepared from neonates produce nearly pure glial populations. The latter system can also be used as a source of pure cultures of astrocytes, oligodendrocytes (*14,15*), and bipotential glial progenitors (*16*).

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