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Compartmentalized neuronal cultures

Armine Darbinyan^{1,2}, Paul Pozniak¹, Nune Darbinian¹, Martyn K. White¹, and Kamel Khalili¹

¹ Department of Neuroscience Temple University School of Medicine Philadelphia, PA 19140

Abstract

The culturing of neurons results in formation of the layer of neurons with random extensive overlapping outgrowth. To understand specific roles of somas, axons and dendrites in complex function of neurons and to identify molecular mechanisms of biological processes in these cellular compartments various methods were developed. We utilized AXon Investigation System (AXISTM) manufactured by Millipore. This device provides an opportunity to orient neuronal outgrowth and spatially isolate neuronal processes from neuronal bodies. AXIS device is a slide-mounted microfluidic system, which consists of four wells. Two of the wells are connected by a channel on each side of the device (Figure 12.1). Channels are connected by microgrooves (approximately 120). The size of microgrooves (10 μ m in width and 5 μ m in height) do not permit passage of cell through while allowing extension of neurites. The microfluidic design also allows an establishment of a hydrostatic gradient when the volume in one chamber is greater than that in the other (1). This feature allows studying of the effect of specific compounds on selected compartments of neurons.

Keywords

Compartment cultures; neurite outgrowth; axonal processes

2. Materials

Isolate human fetal cortical neurons according the protocols described in Chapter 2 (steps 1-6). Carry out all procedures in sterile conditions at room temperature unless otherwise specified.

2.1. Brain tissue

1. Human fetal brain is obtained from Advanced Bioscience Resources (ABR), Inc., Alameda, CA 94501 USA in accordance with NIH guidelines.
2. One timed-pregnant, embryonic day 16 or 17 (ED16 or ED17) Sprague-Dawley rat (Charles River Laboratories). [See Note 1]

²Present address: Mount Sinai Medical Center New York, NY 10029

¹Protocols using human tissue and animals must be approved by an Institutional Animal Care and Use Committee (IACUC).

2.2. Reagents

1. Tryple™ Express (Gibco, cat# 12605) [See Note 2] plus DNase I (10 U/ml).
2. Immunopanning cocktail: 50 mM Tris-HCl, pH 9.5, 1% BSA (w/v), 50 µg/ml anti-PSA-NCAM antibody (Developmental Studies Hybridoma Bank, University of Iowa, DSHB, 5A5).
3. Neuronal medium: Neurobasal medium, B27 supplemented with vitamin A, 0.25 mM Glutamax (Invitrogen), 5 ng/ml BDNF 100 units/ml penicillin and 100 units/ml streptomycin.
4. 70% ethanol
5. 4% paraformaldehyde (PFA) in 1xPBS

2.3. Dissection instruments, plasticware and glassware (all sterile)

1. Dissecting forceps, straight.
2. Dissecting forceps, blunt.
3. Mayo dissecting scissors 5.5 in., Straight.
4. Iris scissors, straight.
5. Iris scissors, curved.
6. Delicate dissecting scissors, straight.
7. Spatula, microdissecting.
8. Handi-Hold* Microspatula.
9. Cell strainers, mesh size 70 µm.
10. 60 mm tissue dishes
11. 100 mm petri dishes (bacterial grade, non TC-treated)
12. Conical tubes 15 ml and 50 ml.
13. Glass slides
14. Cover slips
15. 37°C, 5.0 % CO₂ incubator, 95% humidity
16. Biosafety laminar flow/tissue culture hood

3. Methods

We adjusted protocols provided by the Millipore to our laboratory settings and developed additional steps to insure successful results while using the AXIS device.

²Tryple™ Express is a special formulation of Trypsin/EDTA optimized for the culture of neurons.

3.1. Preparation of slides

This is the one of the most important steps in this protocol. Perform all steps in a tissue culture hood in sterile conditions.

1. Soak the glass slides in 70% ethanol for at least 3 hours in sterile Petri dishes a day before preparation of cells. Soak forceps in 70% ethanol at least 3 hours in sterile glass beaker a day before preparation of cells.
2. Wash slides with sterile water three times.
3. Aspirate excess of water and air dry slides.
4. Transfer a single air dried slide into new petri dish using sterile forceps.
5. Pipette 500 μ l of 0.5 mg/ml poly-D-lysine solution on the slide and place a second sterile air dried slide onto it. Incubate overnight at 4°C. Both glass slides will be coated with poly-D-lysine.
6. Next day gently separate slides using two forceps, rinse with sterile water and air dry at least 2 hours.
7. Place the poly-D-lysine coated slide in new sterile petri dish (coated surface is facing up). The slide is ready to use.

3.2. Assembling the AXIS™ device on slides and preparation for plating of cells

AXIS™ Axon Isolation Devices (Millipore) enable easy isolation, observation and testing of developing neurites. It consists of four wells, two channels, and a set of microgrooves. Two of the wells are interconnected by a channel on each side of the device. The microgrooves are located in the area between the two channels (Fig. 1). All steps should be performed in sterile conditions in the tissue culture hood.

1. Using sterile forceps carefully place AXIS device on the coated surface of the glass slide with the microgroove imprinted side facing down. The AXIS device will adhere easily to the glass. The space will be formed between imprinted surface on the AXIS and the glass slide surface. Device can be carefully repositioned if necessary. It is important to avoid touching imprinted surface to prevent damage to microgrooves and preserve the structural integrity of the device. Designate 2 wells (A and B) for applying cell suspension. Other two (C and D) will be cell free. [See Note 3]
2. Apply gentle pressure to the top surface of the device at various points using blunt forceps. Avoid excessive force, which can compress and deform channels.
3. Add 200 μ l of neuronal media to well A. Avoid bubbles, which can enter the channel and block it. Media should flow through the channel connecting A and B wells and will appear at the ostium of the channel in the well B quickly (during 1-2

³When using several devices in one experiment it may save time and can be convenient to place all devices on the slides in the same orientation.

min). If there is a delay, set the pipette at 100 μ l and pipette up and down 3-4 times with the pipet tip at the ostium of the channel in well A. Avoid bubbles.

4. Add 200 μ l of neuronal media to well B. Incubate 15 min at room temperature.
5. Add 100 μ l of neuronal media to well C. Media should flow through the channel connecting C and D wells and will appear at the ostium of the channel in the well D.
6. Add 100 μ l of neuronal media to well D.

3.3. Plating neurons

1. Count cells using hemacytometer. Prepare 500 μ l cell suspension at a concentration 5-6 million cells per ml in neuronal medium (with 2 ng/ml BDNF).
2. Aspirate the media from all wells.
3. Plate 7 μ l of cell suspension directly into the channel connecting wells A and B and incubate AXIS device with cells for 20 min in tissue culture incubator at 37°C. [Note 4]
4. Add 100 μ l of neuronal media to wells A and B. Add additional 100 μ l of media to wells A and B (total 200 μ l).
5. Add 100 μ l of neuronal media (with 10 ng/ml BDNF) to wells C and D. The BDNF concentration gradient between axonal and somal side stimulate directed growth of neurites
6. Incubate AXIS device containing neuronal cultures in tissue culture incubator at 37°C.
7. Monitor cells closely, add fresh media every second day.

The process of extension into the axonal side chamber can be assessed by immunocytochemistry using anti- β III tubulin antibody after 10-12 days in vitro (Fig. 2).

Recent studies have demonstrated that axons similar to dendrites, are capable of synthesizing new proteins via translation of localized mRNAs (2-6). The importance of local protein synthesis has been established in regenerating axons (5,7,8). Using the AXIS device it is possible to identify the potential roles of proteins and RNA transcripts present in neuronal processes exposed to various conditions (9). The axonal mRNA or protein pool can be compared to total neuronal RNA or protein pool (1,10,11), which can help to obtain important information regarding molecular processes in neurites in response to signals.

References

1. Park JW, Vahidi B, Taylor AM, et al. Microfluidic culture platform for neuroscience research. *Nat Protocols*. 2006; 1:2128–2136.

⁴If necessary, increase the incubation time to avoid displacement of cells. Do not disturb cells and keep AXIS device horizontal to prevent passage of cells through microgrooves to the axonal side.

2. Taylor AM, Berchtold NC, Perreau VM, et al. Axonal mRNA in uninjured and regenerating cortical mammalian axons. *J Neurosci*. 2009; 29:4697–707. [PubMed: 19369540]
3. Sutton MA, Schuman EM. Dendritic protein synthesis, synaptic plasticity, and memory. *Cell*. 2006; 127:49–58. [PubMed: 17018276]
4. Campbell DS, Holt CE. Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. *Neuron*. 2001; 32:1013–1026. [PubMed: 11754834]
5. Wu KY, Hengst U, Cox LJ, et al. Local translation of RhoA regulates growth cone collapse. *Nature*. 2005; 436:1020–1024. [PubMed: 16107849]
6. Lin CH, Forscher P. Cytoskeletal remodeling during growth cone-target interactions. *J Cell Biol*. 1993; 121:1369–1383. [PubMed: 8509456]
7. Willis DE, Twiss JL. The evolving roles of axonally synthesized proteins in regeneration. *Curr Opin Neurobiol*. 2006; 16:111–118. [PubMed: 16418002]
8. Yaron A, Zheng B. Navigating their way to the clinic: emerging roles for axon guidance molecules in neurological disorders and injury. *Dev. Neurobiol*. 2007; 67:1216–1231. [PubMed: 17514715]
9. Quinn CC, Wadsworth WG. Axon guidance: asymmetric signaling orients polarized outgrowth. *Trends Cell Biol*. 2008; 18:597–603. [PubMed: 18951796]
10. Taylor AM, Blurton-Jones M, Rhee SW, et al. A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat Methods*. 2005; 2:599–605. [PubMed: 16094385]
11. Chilton JK. Molecular mechanisms of axon guidance. *Dev Biol*. 2006; 292:13–24. [PubMed: 16476423]

Figure 1.

A. Schematic presentation of AXIS device. B. Phase image of the portion of the assembled device (the black box on panel A), containing the microgrooves and adjacent chambers connected with wells. C. Phase image of the microgrooves.

Figure 2.

A. Neurons immunostained with anti- β III tubulin antibody (green fluorescence). B. DAPI-labeled nuclei (blue) visualized in the somal side chamber. C. Merged image.