Video Article

Preparation and Maintenance of Dorsal Root Ganglia Neurons in Compartmented Cultures

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Abstract

Neurons extend axonal processes that are far removed from the cell body to innervate target tissues, where target-derived growth factors are required for neuronal survival and function. Neurotrophins are specifically required to maintain the survival and differentiation of innervating sensory neurons but the question of how these target-derived neurotrophins communicate to the cell body of innervating neurons has been an area of active research for over 30 years. The most commonly accepted model of how neurotrophin signals reach the cell body proposes that signaling endosomes carry this signal retrogradely along the axon. In order to study retrograde transport, a culture system was originally devised by Robert Campenot, in which cell bodies are isolated from their axons. The technique of preparing these compartmented chambers for culturing sensory neurons recapitulates the selective stimulation of neuron terminals that occurs in vivo following release of target-derived neurotrophins. Retrograde signaling events that require long-range microtubule dependent retrograde transport have important implications for the treatment of neurodegenerative disorders.

Video Link

The video component of this article can be found at http://www.jove.com/details.php?id=951

Protocol

Preparation of reagents

1. *Collagen coating*: collagen coat p35 tissue culture plates and place in an oven at 37°C for 2 days before greasing the dividers. The final concentration of collagen should be at .71 mg/ml diluted in .001N HCl. Then, add 1 ml of mixture per plate.
2. *Grease loaders*: In order to fill the grease loader, a 60mL syringe must first be filled with Corning vacuum grease. Use the syringe to fill the grease loader, wrap it in foil and then autoclave for 45 minutes.
3. *Teflon dividers*: the dividers can be re-used after each experiment but must first be properly cleaned. Remove the divider from the plate, wipe off all of the remaining grease and place in sulfuric acid for 2 days. After removing from the acid, rinse with water 3X, boil for 20 minutes, allow to dry, place in a glass p100 petri dish and autoclave for 20 minutes.
4. *N2-methylcellulose*: Weigh out 1.5g of methylcellulose and place it in a 500mL bottle. Add a stir bar and autoclave it for 20 minutes on dry (from this point all work must be sterile). Next, add 500 mLs of serum free media (N_2), and stir in a cold room until it dissolves. Aliquot into 50 mL conicals and freeze at -20°C. For working stock, aliquot one of the 50 mL conicals into 1mL tubes and freeze at -20°C.
5. *DRG media*: DMEM, 5% Heat inactivated horse serum, and 1% penicillin streptomycin.
6. 100ng/mL DRGN media: The stock concentration of both nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) is 1mg/mL. Dilute each of the neurotrophins 1:10,000 into the DRG media. Cultures can be grown in NGF alone; this alters the complement of neurons that survive in the cultures.

   Note: When needed, the concentration of (cytosine arabinoside) AraC is 1uM and used at a final concentration of 0.3uM. This will inhibit growth of Schwann cells and other glia.

7. *10ng/mL DRGN media*: Dilute the 100ng/mL DRGN media (1:10) with DRG media.

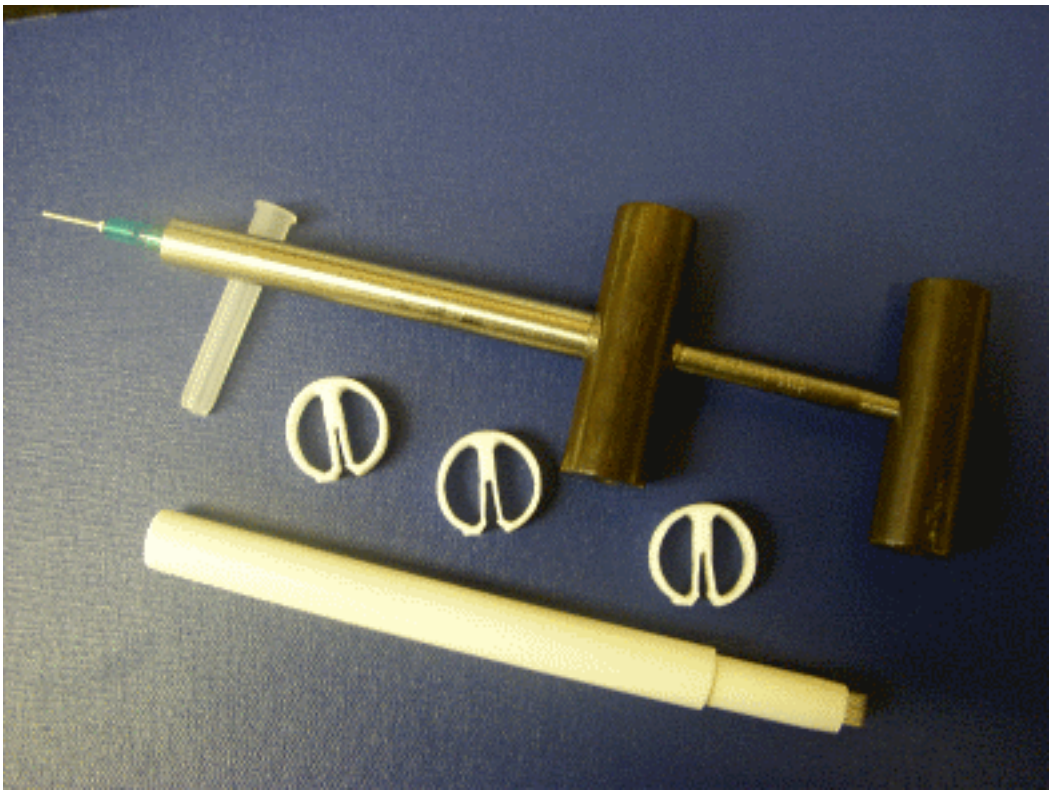

Figure 1. Tools needed for set-up

Setting up the compartmented chambers (start this process 1-2 days before the dissection)

1. Make a scratch in the middle of a collagen coated p35 dish with an outward motion.
2. Place 30ul of N2-methylcellulose on the middle of the scratch. Set dishes aside until divider is greased.
3. Attach a 23-gauge luer stub adapter to the grease loader. Grip the Teflon divider with a pair of 90° angle hemostats and lay it flat with the divider facing up under a microscope. Trace the divider with grease making sure that each time the adapter is placed at a new starting point the adapter is inserted into the grease from the previous step so that there is a continuous line of grease (see diagram). Once the grease is applied to the entire divider, turn one of the prepared p35 dishes upside down and place it so the N2-methylcellulose is over the middle compartment. Press down on the bottom of the dish with a pair of tweezers. Make sure to press on the inside of the divider in four corners (upper left, lower right, upper right, lower left, indicated in diagram by "X").

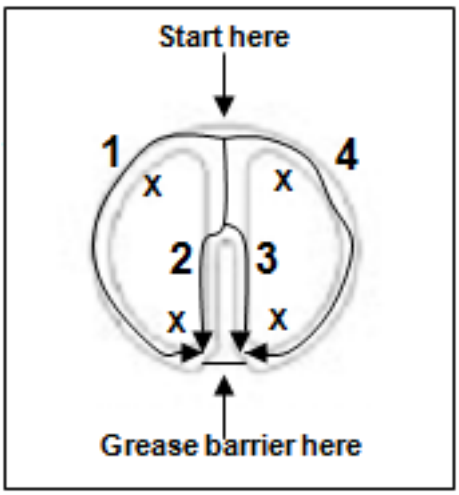

Figure 2: Steps to greasing the divider

Note: It is important to press firmly enough so that the grease makes a complete seal with the dish, but if too much pressure is added, the axons will not cross into the side compartments.

Pick up the hemostats, turn it over and unclamp the divider. Lastly, place the dish with the divider firmly attached under the microscope focusing on the bottom of the middle compartment. With the grease loader, make a small barrier (.25cm) so that once the cells are placed in the middle compartment they cannot leak out.

4. After having set up several cultures, place DRG media in each of the side compartments and place in an incubator in which the cells will be maintained. Allow the cultures to sit for several hours and then check for leakage. If the media has leaked into the middle compartment, then the culture is unusable.

Note: When first learning this technique, it is important to set up more cultures than are needed for an experiment, as several will be leaky.

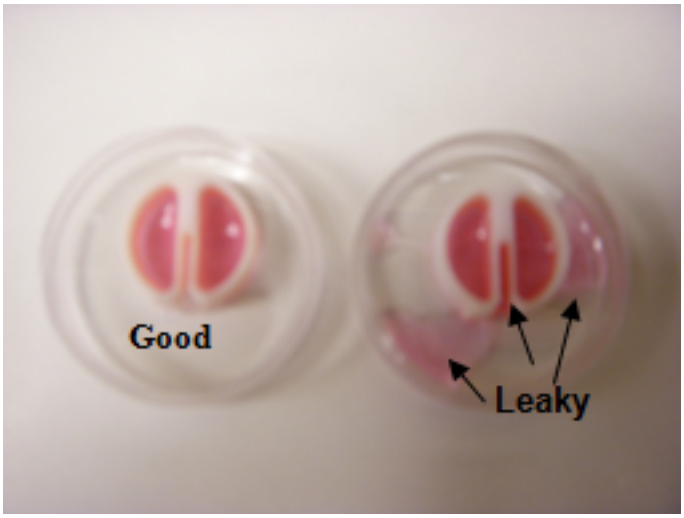

Figure 3: "Good vs leaky" culture

Maintaining DRG neurons in compartmented cultures

1. *Day 1*: Replace DRG media in the side compartments with 100ng/mL DRGN media + AraC. Perform dissection and add cells to center compartment (100,000 cells).
2. *Day 2*: Add 10ng/mL media + AraC to the outside of the Teflon divider until the media flows over the grease barrier and exchanges fluid with the center compartment.
3. *Day 3*: Replace media in the side compartments to 100ng/mL DRGN omitting the AraC and the surround with 10ng/mL DRGN omitting the AraC.
4. *Day 6*: Replace media in the side compartments to 1ng/mL + AraC and the surround with DRG media + AraC.
5. *Day 9*: Use for experimentation.

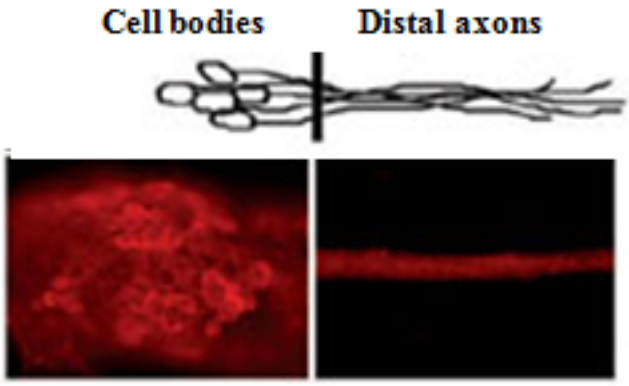

Figure 4: IHC images of cell bodies and distal axons

Note: When changing the media, it is important to aspirate the liquid from the top of the each side compartment. Also, never change the media from the middle compartment itself, only from the surround, and let it flow over the grease barrier into the center.

Discussion

In this video, we have demonstrated how to prepare and maintain compartmented chambers for use in culturing DRG neurons. Done properly, this system allows separation of the cell body from the axon in order to study mechanisms by which neurotrophins signal across long axons. Since there is fluidic isolation between the compartments, it allows for selective stimulation or treatment of one compartment without the other compartments being affected. Compartmented chamber cultures can support other cell types including sympathetic neurons from the superior cervical ganglia, retinal ganglion neurons, and cortical neurons. Spatial understanding of neurotrophin signal transduction may provide novel insights into treatments of neurodegenerative disorders. Several neurodegenerative disorders, including Alzheimer's disease, Huntington's disease and motor neuron disease, are associated with defects in axonal transport. Recent studies have used microfluidic chambers instead of these compartmented chambers. The microfluidic chambers[4,5] have several advantages for imaging analysis.

Prior studies have tested the ability of these cultures to prevent diffusion between the axon and the cell body compartment[1,3,6]. This can easily be tested by adding low concentrations of a dye such as trypan blue to one compartment only, and look for diffusion of the dye. There should be little or no diffusion visible within 24 hours.

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