Science Education Collection

Primary Neuronal Cultures

URL: https://www.jove.com/science-education/5214

Abstract

The complexity of the brain often requires neuroscientists to use a simpler system for experimental manipulations and observations. One powerful approach is to generate a primary culture by dissecting nervous system tissue, dissociating it into single cells, and growing those cells *in vitro*. Primary cultures make neurons and glia easily accessible to the experimental tools required for techniques like genetic manipulation and time-lapse imaging. Furthermore, these cultures represent a highly controllable environment in which to study complex phenomena such as cell-cell interactions.

This video provides an overview of the major steps in producing primary neuronal cultures, which include selecting and dissecting the tissue of interest, mechanically and chemically breaking down the tissue to produce a single cell suspension, plating the cells, and maintaining the cultures in the appropriate media. Several example experiments are also presented to show how cultured cells can be used to investigate protein trafficking, morphological changes, and electrophysiology in living neurons.

Transcript

The brain is one of the most complex organs in the body, so neuroscientists often need a simpler system for experimental manipulations and observations. Cell culturing is one such system that enables easy access to living neurons. In general, culturing cells involves growing them in special solutions called culture media within a sterile, controllable environment – usually a warm incubator. A primary culture is one that is produced from tissue dissected from an organism. Different types of primary neuronal cultures can be made from a variety of animals, developmental stages and nervous system tissues.

This video will provide an introduction to the method of producing primary neuronal cultures and some of the experiments, which commonly use these cells.

Many of the animal models important to neuroscience research can be used to prepare primary neuronal cultures. With mice and rats – two of the most commonly used mammals in biomedical research – neuronal tissue from embryos, newborn pups, and adults can be dissected for culturing. Embryos and perinatal pups are typically preferred since the brain cells are immature and less susceptible to damage.

Various nervous system tissues can be isolated and used to produce different types of primary neuronal cultures. For example, specific parts of the brain can be dissected, such as the cerebellum, cortex and hippocampus. The spinal cord or components of the peripheral nervous system, such as the dorsal root ganglia, can also be dissected and cultured to grow specific types of neurons.

Regardless of the tissue source, the general method for producing primary neuronal cultures can be summarized in a few major steps: Dissection, dissociation, plating, and maintenance.

Let's begin an in-depth review of these steps with the first one: Dissection. In preparing for the dissection and culturing steps, sterility is critical to prevent cultures from becoming contaminated. Tools must be sterilized with alcohol, and a laminar flow hood is often used to remove airborne contaminants.

For culturing rodent neurons, tissue should be removed from freshly euthanized animals into a cold, buffered salt solution. Using a dissecting microscope, smaller parts of the tissue – such as the hippocampi – can be carefully isolated for further processing.

Next, it's necessary to break the sample down into its cellular components in a process known as dissociation. The dissected tissue is first minced using a scalpel or scissors. The resulting tissue pieces are then transferred to a new container. A proteolytic enzyme such as trypsin or papain is then added to digest the extracellular matrix proteins that bind the cells together. Following a short incubation in a warm incubator or water bath, the tissue pieces are gently washed with buffer to remove the enzymes.

The softened tissue pieces can now be dissociated by trituration, which involves passing the tissue through a pipet multiple times so that the cells are freed into a single cell suspension. At this point, the cells can be counted for concentration and checked for viability using stains like Trypan blue, which helps determine how much the suspension should be diluted for successful growth.

At last, the neurons are ready for culture! Let's review some important measures that need to be taken in order to keep them alive and healthy. The composition of the culture media that is used to grow the neurons is very important. Special supplements that support neuronal survival and growth are usually added to the media. Other additives can include drugs that inhibit the division of non-neuronal cells such as glial cells.

After the neuronal cell suspension is mixed with the media, the cells are ready to be plated in the desired containers. These can include culture dishes and plates or glass coverslips placed inside these containers. Since neurons cannot grow directly on glass, the coverslips are treated beforehand to create better surfaces for cell attachment and growth. These treatments can include coating with synthetic extracellular matrix proteins; for example, poly-lysine and laminin; or acid etching to roughen the surface.

Once plated, the neurons are grown inside a warm, humidified incubator. The growth of neuronal processes can be observed within hours to days. To maintain the cultures, a portion of the culture media is replaced with fresh media about once a week. Typically, primary neuronal cultures are used for experiments anywhere from a few days to a few weeks after plating.

Now that you have neurons growing in cultures, what can you do with these cells?

One basic method commonly applied to neuronal cultures is genetic manipulation by transfection or viral transduction. Genetic material encoding, for example, a mutant protein, can be introduced into the cultured neurons to alter neuronal function. Alternatively, transfection with double stranded RNA is an effective technique for blocking protein expression. The availability of multiple methods and reagents for genetic manipulation make this a particularly valuable application for a wide variety of research questions.

An example question that can be addressed with transfections in neuronal cultures is: How and where are certain proteins or protein complexes trafficked to the neuron's various morphological structures? To get at the answer, neurons are transfected with DNA encoding the protein of interest, which can be fused with a fluorescent protein such as green fluorescent protein or GFP. The movement patterns and localization of the fluorescently-tagged protein can then be monitored with live imaging. This method can be used to study, for example, the trafficking of neurotransmitter receptors to the cell surface.

Primary neuronal cultures are also very useful for electrophysiology studies, in which the electrical activity of single cells can be measured using microelectrodes. The single layer of neurons in a culture means that individual cells are easy to target, and manipulations such as pharmacological treatments are quickly and evenly applied. For example, the effect of a test compound on a single neuron's activity can be measured with the patch clamp technique in cultured neurons.

You've just watched JoVE's introduction to primary neuronal cultures. In this video, we've demonstrated how these cultures are prepared and the types of experiments for which they're commonly used. Primary neuronal cultures can be made from a wide variety of tissue sources, typically using a method that involves dissection, dissociation into a cell suspension, and growing in culture media in incubators.

Thanks for watching!