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Explant Culture of Neural Tissue

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Abstract

The intricate structure of the vertebrate nervous system arises from a complex series of events involving cell differentiation, cell migration, and changes in cell morphology. Studying these processes is essential to our understanding of nervous system function as well as our ability to diagnose and treat disorders that result from abnormal development. However, neural tissues are relatively inaccessible for experimental manipulations, especially in embryonic mammals. As a result, many scientists take advantage of explant culture in order to study neurodevelopmental processes in an “organotypic” environment, meaning that the tissue is removed from the organism but its complex cellular architecture is maintained. Generally, explant cultures are created by careful dissection of neural tissue that is then submerged in carefully designed growth media and cultured *in vitro*.

This video will first provide a brief overview of neural explant culture, including its advantages over other *in vitro* methods and important considerations for maintaining healthy tissue. Next, a general protocol will be provided for setting up an explant culture from embryonic mouse brain, outlining the isolation of embryos from the mother and dissection of the brain. The presentation also includes an overview of slice culture, in which thin sections of nervous system tissue are generated for improved visual access to the developing cells. Lastly, a few applications of these techniques will be provided to demonstrate how they can be used to answer important questions in the neurodevelopmental field.

Transcript

Explant cultures serve as a technique to investigate the development of specific cell populations and neural structures. In developmental neuroscience experiments, explants are neural tissues excised from an embryo for continued development *in vitro*. These cultures give researchers the ability to manipulate and visualize the developing tissues in ways that are not possible *in vivo*. This video will introduce some important principles behind working with explanted tissues, step-by-step procedures for two approaches to explant culture, as well as applications of this technique.

Before delving into the methods, lets go over some basic principles. Explants can be established from a number of model organisms and a variety of tissue types. Generally, the cultures are created by carefully removing neural tissue from an embryo, dissecting away a region of interest, and placing it into an artificial environment.

Tissues can also be sectioned into thin sheets and grown in “slice culture.” Because the culture environment is designed to mimic the *in vivo* conditions of the whole organ, this culture strategy is often called “organotypic.”

Before getting started, be sure to sterilize your instruments with 70% ethanol. Next, euthanize a pregnant mouse using your lab’s preferred method. Then, surgically excise the uterus and place in ice-cold buffer. Transfer the dish to a dissection microscope and remove individual embryos from their yolk sac. Next, isolate the brain, carefully dissect out your region of interest, and transfer to a culture dish containing culture medium. Explants can be maintained in a 37 °C incubator containing 5% CO2 for a few weeks by replacing 50% of the medium every 2 - 3 days.

Slice culture of brain tissue requires a few extra steps. Prior to sectioning, the tissue is embedded in agarose, which provides support to the tissue so it remains intact while it’s being sliced. To do this, a 1.5% low melting point agarose solution is heated until the agarose dissolves. Next, the agarose is transferred to embedding molds and allowed to cool slightly to avoid damaging the tissue.

The tissue can then be carefully submerged and the agarose left to harden. The resulting blocks are trimmed and then glued to a specimen stage and sectioned using a vibratome, which is an instrument that uses a vibrating blade to cut thin slices of living tissue. As slices are generated, they are carefully transferred to a coated plate containing culture media and cultured as previously mentioned.

There are a number of advantages to using explant cultures over *in vivo* and other *in vitro* methods. First, cells in explanted tissue are more accessible to experimental tools. Second, the fact that explants maintain the complex cellular architecture of developing neural tissue means that cell-cell interactions can be studied. Third, since they can control the chemical composition of the culture medium, scientists can use explants to test the effect of specific compounds on tissue development.

Nevertheless, since it’s being removed from its natural environment, special care must be taken to maintain happy and healthy tissue *in vitro*. For example, the presence of extracellular matrix, or ECM, has a significant impact on cell behavior, so purified ECM proteins are often used to coat culture dishes. Another important consideration is the solution in which the explants are bathed. While traditional cell culture media is often used, some experiments require solutions that closely resemble the fluid circulating in the central nervous system: the cerebrospinal fluid, which is a critical reagent in experiments like the ones you are about to see.

Now that we have gone over explant culture methods, lets see how these techniques are used.

Cell migration assays use explanted tissue to examine the repulsive and attractive signals that are involved in neural cell movement. In this experiment, beads that have been previously soaked in growth factors are implanted into hindbrain explants to examine neural cell migration. After 3 - 4 days of exposure, neurons were imaged using a confocal microscope. The results show that motor neurons migrate towards beads soaked in vascular endothelial growth factor, but not control beads soaked in buffer.

Co-culture assays are often used to investigate cell-cell interactions during development. In this example, segments of spinal cord were cultured on top of a layer of muscle cells to study how connections are made between spinal motor neurons and skeletal muscle. As early as 2 days after incubation, projections from the neurons, also known as neurites, are seen emerging from the explant. Within 5 days, functional innervation is observed by the contraction of the muscle cell layer.

During development of the nervous system, neurons must elongate their axons to establish a connection between the target tissue and the central nervous system. One way of studying this complex process is through axon guidance assays. Researchers use explanted tissues to examine the factors within the neurons and in the surrounding environment that help guide the axon to its proper location.

You've just watched JoVE's guide to explant culture of neural tissue. This video covered an overview of the advantages of explant cultures, culture strategies, step-by-step protocols of two commonly used explant procedures and ways these techniques are used in the lab today.

Thanks for watching!