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Explant Culture for Developmental Studies

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Abstract

Explant culture is a technique in which living cells or tissues are removed from an embryo for continued development outside of the organism. This *ex vivo* approach allows researchers to manipulate and observe developing tissues in ways that are not possible *in vivo*. Once established, explant culture is frequently used to understand the role of genes and signaling molecules in organogenesis.

This video will first introduce the basic principles of explant culture and demonstrate a protocol to isolate and grow explanted mammalian tissues. Common genetic and molecular methods of manipulating explant cultures will then be discussed. Finally, the viewers will learn about how explant techniques are currently being applied to study organ development.

Transcript

Explant culture is a technique in which whole tissues and organs are removed, or "explanted," from a fetus or embryo and are cultured out of the body, or "*ex vivo*." These methods provide a unique window to help us understand the process of development by enabling direct observations of organ and tissue growth—a process called organogenesis.

This video will examine the basic principles of explant culture, outline key steps of the procedure, discuss typical manipulations, and provide specific applications of this technique in developmental studies.

Before describing how to culture explants, let's review some of the principles behind this technique.

Explant culture is applicable to a variety of embryonic tissues from a broad range of organisms. This technique is ideal for the study of organ development, as structural changes, known as morphogenesis, can be directly observed as a function of time. In addition, tissues can be treated with experimental molecules to determine their impact on development. Moreover, when explant culture is combined with molecular tools to alter gene expression and fluorescence microscopy, it becomes a powerful tool for addressing questions of cell and tissue differentiation.

Next, let's review a protocol for explant culture of mammalian tissues.

To start the procedure, embryos are removed from a euthanized pregnant rodent. After removal of the surrounding membranes, the embryo is isolated. The tissues or organs of interest are then isolated from the extracted embryo and transferred to plates for growth. Media containing antibiotics are added and the plates are grown in an incubator set for 37°C. Tissue explants are now ready for manipulation.

Once explant cultures are established, several types of manipulations can be performed.

For example, the explanted tissue can be genetically manipulated to test the role of a gene of interest on a specific developmental process. This can be achieved by introducing genetic material into the tissue using techniques like electroporation, in which an electrical field is used to drive injected DNA into nearby cells.

Explants are also frequently used to test the roles that signaling molecules, like growth factors, play in organogenesis. Tissue cultured *in vitro* is especially useful for these types of studies because treating with the compound is as simple as adding it to the culture media. However, small beads soaked in experimental compounds can also be implanted into the tissue for a more controlled approach to chemical treatment.

Regardless of the approach, a major advantage of the explant technique is that the effects of experimental manipulations can be easily visualized in real time, using light or fluorescent microscopy.

Now, let's look at some specific applications of *ex vivo* culture.

First, explants represent a model system in which tissue development can be directly visualized. For example, these researchers removed the developing pancreas from embryonic mice and cultured it on glass-bottom dishes for improved imaging.

Development of the tissue can be observed by light microscopy, but the use of transgenic tissue expressing fluorescent markers provides an even more detailed view of developmental processes, like tubule budding. Explanted tissue can also be subjected to immunostaining, which allows researchers to monitor the development of specific cell types, like the insulin-producing cells shown here in blue and green.

Another application of explant cultures is to help decipher the role of specific molecules, like growth factors, in organogenesis.

Here, fetal lung explants dissected from E12.5 mouse embryos were grown in the presence or absence of a growth factor for up to 48 hours. Results indicate that this growth factor inhibits airway formation in the fetal lung.

Finally, explants from early embryos can be used to determine the mechanisms controlling gene expression during the first stages of embryogenesis.

These researchers explanted two different cell types from the 16-cell stage of frog embryos: those that are destined to form dorsal structures, and those that will contribute to ventral structures. The explants were allowed to develop for about a day prior to staining to characterize the expression pattern of a gene specific to dorsal tissue.

Since expression of this gene was observed only in explants from the dorsal region, it was concluded that gene expression programs were encoded in these embryonic cells, rather than being directed by cell-cell interactions that occur later in development.

We have now viewed explant culture systems and observed how it can be used in developmental studies. This technique is a powerful tool that facilitates our understanding of the molecular mechanisms underlying animal development. Thanks for watching!