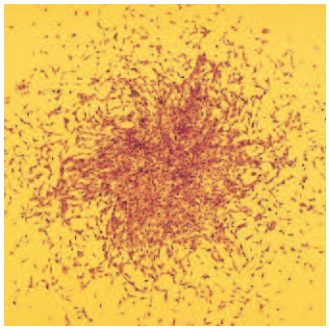
Introduction to Animal Cell Culture

Technical Bulletin



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Introduction

Cell culture has become one of the major tools used in the life sciences today. This guide is designed to serve as a basic introduction to animal cell culture. It is appropriate for laboratory workers who are using it for the first time, as well as for those who interact with cell culture researchers and who want a better understanding of the key concepts and terminology in this interesting and rapidly growing field.

What is Cell and Tissue Culture?

Tissue Culture is the general term for the removal of cells, tissues, or organs from an animal or plant and their subsequent placement into an artificial environment conducive to growth. This environment usually consists of a suitable glass or plastic culture vessel containing a liquid or semi-solid medium that supplies the nutrients essential for survival and growth. The culture of whole organs or intact organ fragments with the intent of studying their continued function or development is called **Organ Culture**. When the cells are removed from the organ fragments prior to, or during cultivation, thus disrupting their normal relationships with neighboring cells, it is called **Cell Culture**.

Although animal cell culture was first successfully undertaken by Ross Harrison in 1907, it was not until the late 1940's to early 1950's that several developments occurred that made cell culture widely available as a tool for scientists. First, there was the development of antibiotics that made it easier to avoid many of the contamination problems that plagued earlier cell culture attempts. Second was the development of

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the techniques, such as the use of trypsin to remove cells from culture vessels, necessary to obtain continuously growing cell lines (such as HeLa cells). Third, using these cell lines, scientists were able to develop standardized, chemically defined culture media that made it far easier to grow cells. These three areas combined to allow many more scientists to use cell, tissue and organ culture in their research.

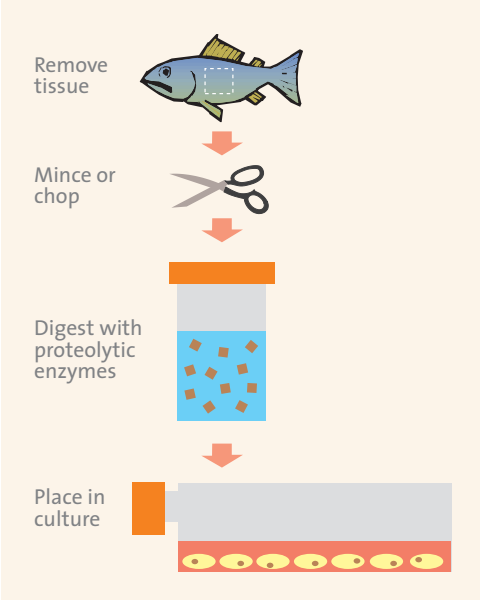
During the 1960's and 1970's, commercialization of this technology had further impact on cell culture that continues to this day. Companies, such as Corning, began to develop and sell disposable plastic and glass cell culture products, improved filtration products and materials, liquid and powdered tissue culture media, and laminar flow hoods. The overall result of these and other continuing technological developments has been a widespread increase in the number of laboratories and industries using cell culture today.

Additional cell culture terminology and usage information can be found on the Society for In Vitro Biology web site at **www.sivb.org/edu_terminology.asp.**

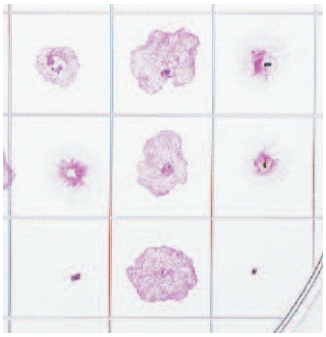
How Are Cell Cultures Obtained?

Primary Culture

When cells are surgically removed from an organism and placed into a suitable culture environment, they will attach, divide and grow. This is called a **Primary Culture**. There are two basic methods for doing this. First, for **Explant Cultures**, small pieces of tissue are attached to a glass or treated plastic culture vessel and bathed in culture medium. After a few days, individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow. The second, more widely used method, speeds up this process by adding digesting (proteolytic) enzymes, such as trypsin or collagenase, to the tissue fragments to dissolve the cement holding the cells together. This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide. This method is called **Enzymatic Dissociation**.



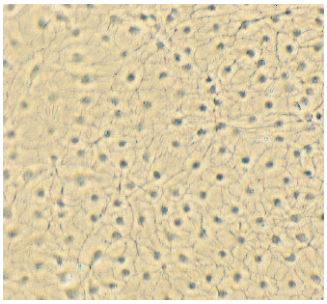
Enzymatic Dissociation


Fixed and stained human foreskin explants on the surface of a 150 mm culture dish. The explants were cultured for approximately two weeks. Two of the nine explants (bottom left and right corners) failed to grow. The remaining explants show good growth. Each square is approximately 2 cm across.

Subculturing

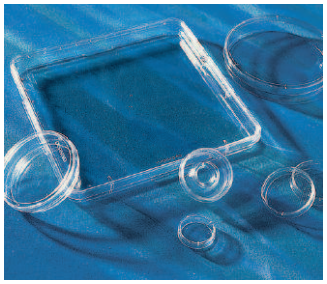
When the cells in the primary culture vessel have grown and filled up all of the available culture substrate, they must be **Subcultured** to give them room for continued growth. This is usually done by removing them as gently as possible from the substrate with enzymes. These are similar to the enzymes used in obtaining the primary culture and are used to break the protein bonds attaching the cells to the substrate. Some cell lines can be harvested by gently scraping the cells off the bottom of the culture vessel. Once released, the cell suspension can then be subdivided and placed into new culture vessels.

Once a surplus of cells is available, they can be treated with suitable cryoprotective agents, such as dimethylsulfoxide (DMSO) or glycerol, carefully frozen and then stored at cryogenic temperatures (below -130°C) until they are needed. The theory and techniques for cryopreserving cells are covered in the Corning Technical Bulletin: General Guide for Cryogenically Storing Animal Cell Cultures (Ref. 9).


Primary culture from the fish *Poeciliopsis lucida*. Embryos were minced and dissociated with a trypsin solution. These cells were in culture for about 1 week and have formed a confluent monolayer.

Buying And Borrowing

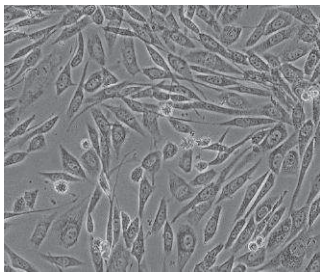
An alternative to establishing cultures by primary culture is to buy established cell cultures from organizations such as the ATCC (www.atcc.org), or the Coriell Institute for Medical Research (ccr.coriell.org). These two nonprofit organizations provide high quality cell lines that are carefully tested to ensure the authenticity of the cells.



Corning culture dishes are available in a variety of sizes and shapes for growing anchorage-dependent cells.

Corning culture flasks are used for growing anchorage-dependent cells.

Corning spinner vessels are used for growing anchorage-independent cells in suspension.



Fibroblast-like 3T3 cells derived from mouse embryos

More frequently, researchers will obtain (borrow) cell lines from other laboratories. While this practice is widespread, it has one major drawback. There is a high probability that the cells obtained in this manner will not be healthy, useful cultures. This is usually due to previous mix-ups or contamination with other cell lines, or the result of contamination with microorganisms such as mycoplasmas, bacteria, fungi or yeast. These problems are covered in detail in a Corning Technical Bulletin: Understanding and Managing Cell Culture Contamination (Ref. 7).

What Are Cultured Cells Like?

Once in culture, cells exhibit a wide range of behaviors, characteristics and shapes. Some of the more common ones are described below. John Paul discusses these issues in detail in Chapter 3 of *Cell and Tissue Culture* (Ref. 3).

Cell Culture Systems

Two basic culture systems are used for growing cells. These are based primarily upon the ability of the cells to either grow attached to a glass or treated plastic substrate (**Monolayer Culture Sytems**) or floating free in the culture medium (**Suspension Culture Systems**).

Monolayer cultures are usually grown in tissue culture treated dishes, T-flasks, roller bottles, CellSTACK® Culture Chambers, or multiple well plates, the choice being based on the number of cells needed, the nature of the culture environment, cost and personal preference.

Suspension cultures are usually grown either:

1. In magnetically rotated spinner flasks or shaken Erlenmeyer flasks where the cells are kept actively suspended in the medium;
2. In stationary culture vessels such as T-flasks and bottles where, although the cells are not kept agitated, they are unable to attach firmly to the substrate.

Many cell lines, especially those derived from normal tissues, are considered to be **Anchorage-Dependent,** that is, they can only grow when attached to a suitable substrate.

Some cell lines that are no longer considered normal (frequently designated as **Transformed Cells)** are frequently able to grow either attached to a substrate or floating free in suspension; they are **Anchorage-Independent.** In addition, some normal cells, such as those found in the blood, do not normally attach to substrates and always grow in suspension.

Types of Cells

Cultured cells are usually described based on their morphology (shape and appearance) or their functional characteristics. There are three basic morphologies:

1. Epithelial-like: cells that are attached to a substrate and appear flattened and polygonal in shape.
2. Lymphoblast-like: cells that do not attach normally to a substrate but remain in suspension with a spherical shape.
3. Fibroblast-like: cells that are attached to a substrate and appear elongated and bipolar, frequently forming swirls in heavy cultures.

It is important to remember that the culture conditions play an important role in determining shape and that many cell cultures are capable of exhibiting multiple morphologies.

Using cell fusion techniques, it is also possible to obtain hybrid cells by fusing cells from two different parents. These may exhibit characteristics of either parent or both parents. This technique was used in 1975 to create cells capable of producing custom tailored monoclonal antibodies. These hybrid cells (called **Hybridomas**) are formed by fusing two different but related cells. The first is a spleen-derived lymphocyte that is capable of producing the desired antibody. The second is a rapidly dividing myeloma cell (a type of cancer cell) that has the machinery for making antibodies but is not programmed to produce any antibody. The resulting hybridomas can produce large quantities of the desired antibody. These antibodies, called **Monoclonal Antibodies** due to their purity, have many important clinical, diagnostic, and industrial applications with a yearly value of well over a billion dollars.