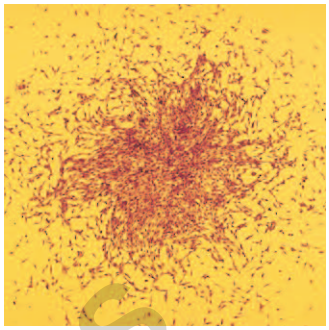
Introduction to Animal Cell Culture

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.

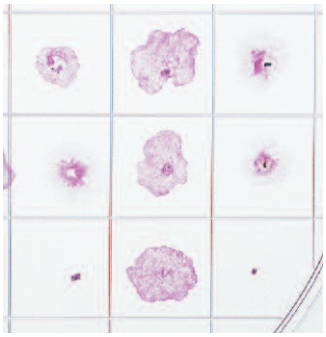
During the 1960's and 1970's, commercialization of this technology had further impact on cell culture that continues to this day. Companies, such as Corning, began to develop and sell disposable plastic and glass cell culture products, improved filtration products and materials, liquid and powdered tissue 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.**

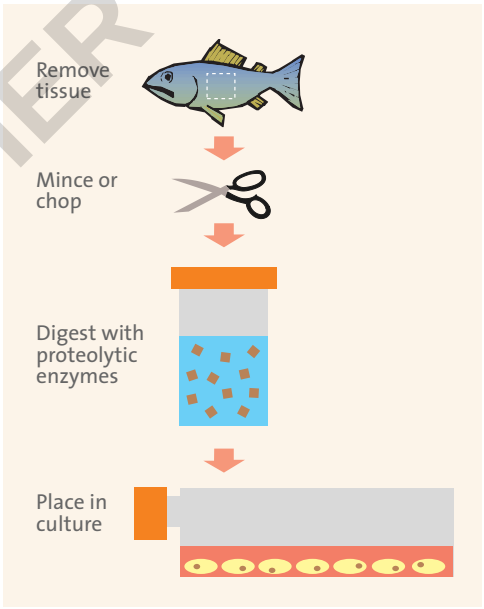
How Are Cell Cultures Obtained?

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**.



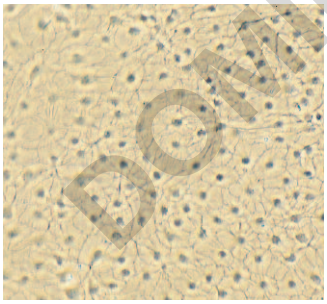
Fixed and stained human foreskin explants on the surface of a 150 mm culture dish. The explants were cultured for approximately two weeks. Two of the nine explants (bottom left and right corners) failed to grow. The remaining explants show good growth. Each square is approximately 2 cm across.



Enzymatic Dissociation

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.



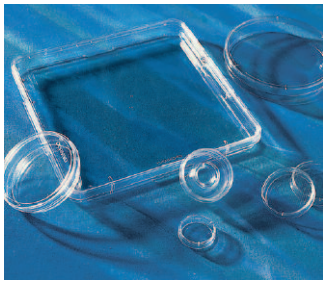
Primary culture from the fish *Poeciliopsis lucida*. Embryos were minced and dissociated with a trypsin solution. These cells were in culture for about 1 week and have formed a confluent monolayer.

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).

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.

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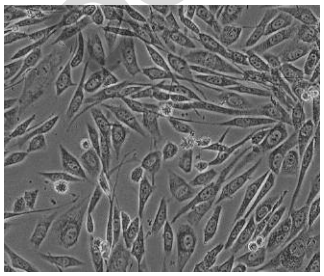
Corning culture dishes are available in a variety of sizes and shapes for growing anchorage-dependent cells.



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.

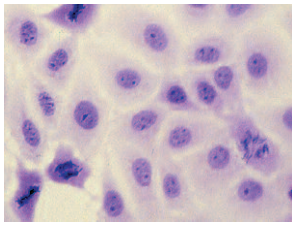
It is important to remember that the culture conditions play an important role in determining shape and that many cell cultures are capable of exhibiting multiple morphologies.

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.

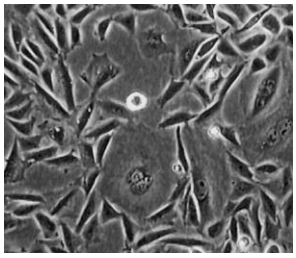
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Functional Characteristics

The characteristics of cultured cells result from both their origin (liver, heart, etc.) and how well they adapt to the culture conditions. Biochemical markers can be used to determine if cells are still carrying on specialized functions that they performed in vivo (e.g., liver cells secreting albumin). Morphological or ultrastructural markers can also be examined (e.g., beating heart cells). Frequently, these characteristics are either lost or changed as a result of being placed in an artificial environment. Some cell lines will eventually stop dividing and show signs of aging. These lines are called **Finite**. Other lines are, or become immortal; these can continue to divide indefinitely and are called **Continuous** cell lines. When a "normal" finite cell line becomes immortal, it has undergone a fundamental irreversible change or "transformation". This can occur spontaneously or be brought about intentionally using drugs, radiation or viruses. **Transformed Cells** are usually easier and faster growing, may often have extra or abnormal chromosomes and frequently can be grown in suspension. Cells that have the normal number of chromosomes are called **Diploid** cells; those that have other than the normal number are **Aneuploid**. If the cells form tumors when they are injected into animals, they are considered to be **Neoplastically Transformed**.



Epithelial-like cell line (Cl-9) derived from rat liver. The mitotic cells indicates this culture is actively growing.



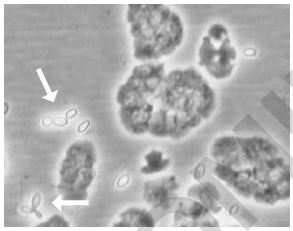
CHO-K1 cells – a widely used continuous (transformed) cell line derived from adult Chinese hamster ovary tissue in 1957.

What Are Some of the Problems Faced by Cultured Cells?

Avoiding Contamination

Cell culture contamination is of two main types: chemical and biological. Chemical contamination is the most difficult to detect since it is caused by agents, such as endotoxins, plasticizers, metal ions or traces of chemical disinfectants, that are invisible. The cell culture effects associated with endotoxins are covered in detail in the Technical Bulletin: Endotoxins and Cell Culture (Ref. 10). Biological contaminants in the form of fast growing yeast, bacteria and fungi usually have visible effects on the culture (changes in medium turbidity or pH) and thus are easier to detect (especially if antibiotics are omitted from the culture medium). However, two other forms of biological contamination, mycoplasmas and viruses, are not easy to detect visually and usually require special detection methods.

There are two major requirements to avoiding contamination. First, proper training in and use of good aseptic technique on the part of the cell culturist. Second, properly designed, maintained and sterilized equipment, plasticware, glassware, and media. The careful and selective (limited) use of antibiotics designed for use in tissue culture can also help avoid culture loss due to biological contamination. These concepts are covered in detail in a Corning Technical Bulletin: Understanding and Managing Cell Culture Contamination (Ref. 7).



Photomicrograph of a low level yeast infection in a liver cell line (PLHC-1, ATCC # CRL-2406). Budding yeast cells can been seen in several areas (arrows). At this low level of contamination, no medium turbidity would be seen; however, in the absence of antibiotics, the culture medium will probably become turbid within a day.

Finding A "Happy" Environment

To cell culturists, a "happy" environment is one that does more than just allow cells to survive in culture. Usually, it means an environment that, at the very least, allows cells to increase in number by undergoing cell division (mitosis). Even better, when conditions are just right, some cultured cells will express their "happiness" with their environment by carrying out important in vivo physiological or biochemical functions, such as muscle contraction or the secretion of hormones and enzymes. To provide this environment, it is important to provide the cells with the appropriate temperature, a good substrate for attachment, and the proper culture medium. Many of the issues and problems associated with keeping cells "happy" are covered in the Corning Technical Bulletin: General Guide for Identifying and Correcting Common Cell Culture Growth and Attachment Problems (Ref. 8).

Temperature is usually set at the same point as the body temperature of the host from which the cells were obtained. With cold-blooded vertebrates, a temperature range of 18° to 25°C is suitable; most mammalian cells require 36° to 37°C. This temperature range is usually maintained by use of carefully calibrated, and frequently checked, incubators.

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Basic environmental Requirements for "Happy" Cells:

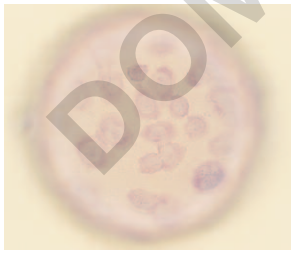
- Controlled temperature
- Good substrate for cell attachment
- Appropriate medium and incubator that maintains the correct pH and osmolality



Corning® Transwell permeable supports are used to study cell transport and migration.



Suspension and microcarrier cultures can be grown in glass and plastic spinner vessels.



CHO-K1 cells growing on a microcarrier bead

Anchorage-dependent cells also require a good substrate for attachment and growth. Glass and specially treated plastics (to make the normally hydrophobic plastic surface hydrophilic or wettable) are the most commonly used substrates. However, **Attachment Factors**, such as collagen, gelatin, fibronectin and laminin, can be used as substrate coatings to improve growth and function of normal cells derived from brain, blood vessels, kidney, liver, skin, etc. Often normal anchorage-dependent cells will also function better if they are grown on a permeable or porous surface. This allows them to polarize (have a top and bottom through which things can enter and leave the cell) as they do in the body. Transwell® inserts are Corning vessels with membrane-based permeable supports that allow these cells to develop polarity and acquire the ability to exhibit special functions such as transport. Many specialized cells can only be truly "happy" (function normally) when grown on a porous substrate in serum-free medium with the appropriate mixture of growth and attachment factors.

Cells can also be grown in suspension on beads made from glass, plastic, polyacrylamide and cross-linked dextran molecules. This technique has been used to enable anchorage-dependent cells to be grown in suspension culture systems and is increasingly important for the manufacture of cell-based biologicals.

The culture medium is the most important and complex factor to control in making cells "happy". Besides meeting the basic nutritional requirement of the cells, the culture medium should also have any necessary growth factors, regulate the pH and osmolality, and provide essential gases (O_2 and CO_2). The 'food' portion of the culture medium consists of amino acids, vitamins, minerals, and carbohydrates. These allow the cells to build new proteins and other components essential for growth and function as well as providing the energy necessary for metabolism. For additional information on this topic, see the article: Construction of Tissue Culture Media by C. Waymouth in *Growth, Nutrition and Metabolism of Cells in Culture*, Volume 1, (1972; Ref. 5).

The growth factors and hormones help regulate and control the cells' growth rate and functional characteristics. Instead of being added directly to the medium, they are often added in an undefined manner by adding 5 to 20% of various animal sera to the medium. Unfortunately, the types and concentration of these factors in serum vary considerably from batch to batch. This often results in problems controlling growth and function. When growing normal functional cells, sera are often replaced by specific growth factors.

The medium also controls the pH range of the culture and buffers the cells from abrupt changes in pH. Usually a CO_2-bicarbonate based buffer or an organic buffer, such as HEPES, is used to help keep the medium pH in a range from 7.0 to 7.4 depending on the type of cell being cultured. When using a CO_2-bicarbonate buffer, it is necessary to regulate the amount of CO_2 dissolved in the medium. This is usually done using an incubator with CO_2 controls set to provide an atmosphere with between 2% and 10% CO_2 (for Earle's salts-based buffers). However, some media use a CO_2-bicarbonate buffer (for Hanks' salts-based buffers) that requires no additional CO_2, but it must be used in a sealed vessel (not dishes or plates). For additional information on this topic, see the article: The Gaseous Environment of the Cell in Culture by W.F. McLimans in *Growth, Nutrition and Metabolism of Cells in Culture* (1972; Ref. 5).

Finally, the osmolality (osmotic pressure) of the culture medium is important since it helps regulate the flow of substances in and out of the cell. It is controlled by the addition or subtraction of salt in the culture medium. Evaporation of culture media from open culture vessels (dishes, etc.) will rapidly increase the osmolality resulting in stressed, damaged or dead cells. For open (not sealed) culture systems, incubators with high humidity levels to reduce evaporation are essential. For additional information, see article by C. Waymouth: Osmolality of Mammalian Blood and of Media for Culture of Mammalian Cells (1970; Ref. 6).

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How to Decide if Cultured Cells Are "Happy"

Evaluating the general health or "happiness" of a culture is usually based on four important cell characteristics: morphology, growth rate, plating efficiency and expression of special functions. These same characteristics are also widely used in evaluating experimental results.

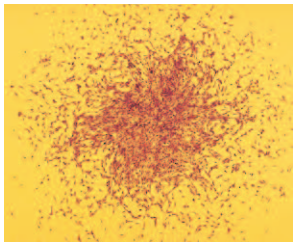


Examining the morphology of these stained roller bottles (containing MRC-5 human fibroblasts) is a good way to check if the cells are "happy". The bottle on the left was rotated at too high a speed resulting in poor attachment and growth and very "unhappy" cells.

The **Morphology** or cell shape is the easiest to determine but is often the least useful. While changes in morphology are frequently observed in cultures, it is often difficult to relate these observations to the condition that caused them. It is also a very difficult characteristic to quantify or to measure precisely.

Often, the first sign that something is wrong with a culture occurs when the cells are microscopically examined and poor or unusual patterns of cell attachment or growth are observed. When problems are suspected, staining the culture vessels with crystal violet or other simple histological stains may show growth patterns indicating a problem. These growth problems are discussed in detail in the Corning Technical Bulletin: General Guide for Identifying and Correcting Common Cell Culture Growth and Attachment Problems (Ref. 8).

Cell counting and other methods for estimating cell number, on the other hand, allow the determination of the **Growth Rate**, which is sensitive to major changes in the culture environment. This allows the design of experiments to determine which set of conditions (culture media, substrate, serum, plasticware) is better for the cells, i.e., the conditions producing the best growth rate. These same or similar techniques can also be used to measure cell survival or death and are often used for in vitro cytotoxicity assays.



A colony of fixed and stained human fibroblast cells

Plating Efficiency is a testing method where small numbers of cells (20 to 200) are placed in a culture vessel and the number of colonies they form is measured. The percentage of cells forming colonies is a measure of survival, while the colony size is a measure of growth rate. This testing method is similar in application to growth rate analysis but is more sensitive to small variations in culture conditions.

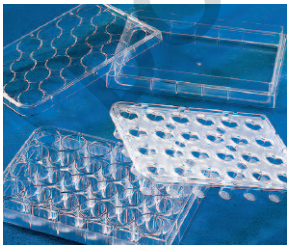
The final characteristic, the **Expression of Specialized Functions**, is usually the most difficult to observe and measure. Usually biochemical or immunological assays and tests are used. While cultured cells may grow very well in subobtimal conditions, highly specialized functions usually require near perfect culture conditions and are often quickly lost when cells are placed in culture.

What is Cell Culture Used For?

Cell culture has become one of the major tools used in cell and molecular biology. Some of the important areas where cell culture is currently playing a major role are briefly described below:

Model Systems

Cell cultures provide a good model system for studying 1) basic cell biology and biochemistry, 2) the interactions between disease-causing agents and cells, 3) the effects of drugs on cells, 4) the process and triggers for aging, and 5) nutritional studies.



HTS Transwell®-24 plates are used for toxicity testing and drug transport studies

Toxicity Testing

Cultured cells are widely used alone or in conjunction with animal tests to study the effects of new drugs, cosmetics and chemicals on survival and growth in a wide variety of cell types. Especially important are liver- and kidney-derived cell cultures.

Cancer Research

Since both normal cells and cancer cells can be grown in culture, the basic differences between them can be closely studied. In addition, it is possible, by the use of chemicals, viruses and radiation, to convert normal cultured cells to cancer causing cells. Thus, the mechanisms that cause the change can be studied. Cultured cancer cells also serve as a test system to determine suitable drugs and methods for selectively destroying types of cancer.

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Corning® roller bottles are widely used for producing viral vaccines.



The Corning CellCube® bioreactor system is ideal for mass production of anchorage-dependent cells.



Corning® Erlenmeyer flasks are often used for growing insect cells in suspension.



Corning microplates are widely used for drug screening.

Virology

One of the earliest and major uses of cell culture is the replication of viruses in cell cultures (in place of animals) for use in vaccine production. Cell cultures are also widely used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms.

Cell-Based Manufacturing

While cultured cells can be used to produce many important products, three areas are generating the most interest. The first is the large-scale production of viruses for use in vaccine production. These include vaccines for polio, rabies, chicken pox, hepatitis B and measles. Second, is the large-scale production of cells that have been genetically engineered to produce proteins that have medicinal or commercial value. These include monoclonal antibodies, insulin, hormones, etc. Third, is the use of cells as replacement tissues and organs. Artificial skin for use in treating burns and ulcers is the first commercially available product. However, testing is underway on artificial organs such as pancreas, liver and kidney. A potential supply of replacement cells and tissues may come out of work currently being done with both embryonic and adult stem cells. These are cells that have the potential to differentiate into a variety of different cell types. It is hoped that learning how to control the development of these cells may offer new treatment approaches for a wide variety of medical conditions.

Genetic Counseling

Amniocentesis, a diagnostic technique that enables doctors to remove and culture fetal cells from pregnant women, has given doctors an important tool for the early diagnosis of fetal disorders. These cells can then be examined for abnormalities in their chromosomes and genes using karyotyping, chromosome painting and other molecular techniques.

Genetic Engineering

The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a major tool to molecular biologists wishing to study the cellular effects of the expression of theses genes (new proteins). These techniques can also be used to produce these new proteins in large quantity in cultured cells for further study. Insect cells are widely used as miniature cells factories to express substantial quantities of proteins that they manufacture after being infected with genetically engineered baculoviruses.

Gene Therapy

The ability to genetically engineer cells has also led to their use for gene therapy. Cells can be removed from a patient lacking a functional gene and the missing or damaged gene can then be replaced. The cells can be grown for a while in culture and then replaced into the patient. An alternative approach is to place the missing gene into a viral vector and then "infect" the patient with the virus in the hope that the missing gene will then be expressed in the patient's cells.

Drug Screening and Development

Cell-based assays have become increasingly important for the pharmaceutical industry, not just for cytotoxicity testing but also for high throughput screening of compounds that may have potential use as drugs. Originally, these cell culture tests were done in 96 well plates, but increasing use is now being made of 384 and 1536 well plates.

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