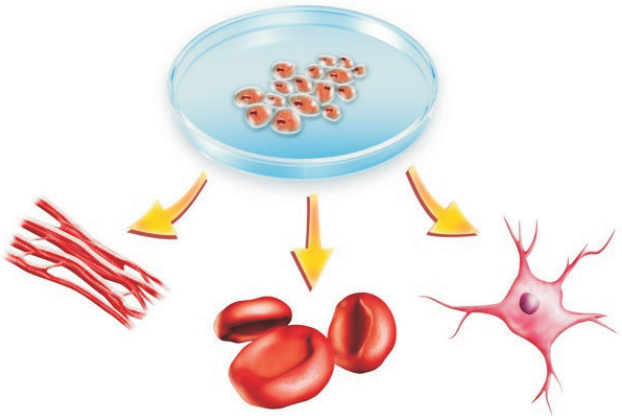
Working with Cell Cultures

EMBRYONIC STEM CELLS may provide answers to many disease situations. We cannot determine their full potential until much more research is done. One of the problems that has occurred is that the stem cells would not grow well in culture. They were able to grow when placed on a layer of mouse "nurse" cells. However, some mouse proteins passed from the mouse cells into the stem cells. The stem cells then could not be used for some research because they were not pure. Being able to culture or grow the types of cells we need for research is very important.



Objective:

Describe how to isolate, maintain, quantify, and store cell cultures.

Key Terms:

aseptic techniques
callus
cell culture
cell suspension
centrifuge
colony
hemacytometer
immortal
isolate
lyophilization
media
reculturing
senescence
streak plate

Methods for Working with Cell Cultures

A very important tool in research is the ability to work with one type of cell at a time. To do so, it is necessary to **isolate**, or separate, cells to obtain the desired cell type. The method used to isolate depends on the type of cells used. Once a cell type is isolated, it is used to establish a **cell culture**, a growing population of the same type of cells. The culture must be maintained and kept pure if research is to be conducted. To know when processes are working or not

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working, the culture must be quantified, or counted, frequently. If research is to go on over long periods or if cultures must be retained for future research, storage methods are required.

ISOLATING CELL CULTURES

Microbe Isolation

Isolation of microbes is difficult because the individual cells are so small. A **streak plate** is a culture made by diluting the microbes through successive streaks. Microbes can be picked up on a sterile transfer loop, and several streaks made along one edge of a sterile agar plate. On the third or fourth streak, separate colonies should develop. Each **colony**, or mass of microbes, is from one original cell reproducing. Once separate colonies are established, they can be picked up using **aseptic techniques**, or sterile methods, and transferred to sterile media to establish cell cultures. **Media** are the water and nutrient materials used to grow cells.

You can also separate colonies from liquid cultures by dilution. A liquid culture often has many microbial cells. If the culture is diluted before it is transferred, the cells will be separated. A microbe may have such specific growth requirements that it can be separated on a selective medium. This is a medium that allows for the growth of the desired microbe but inhibits or prevents the growth of others.

FIGURE 1. Cell culture being observed. (Courtesy, CDC)

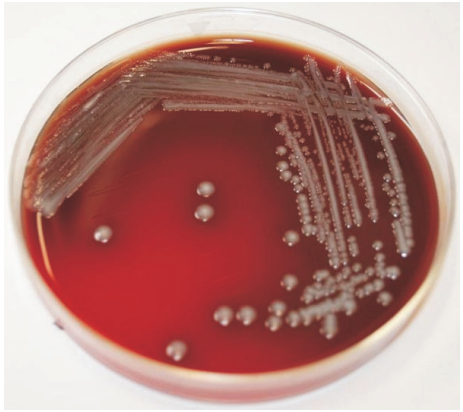

FIGURE 2. Streak plate results. (Courtesy, CDC)

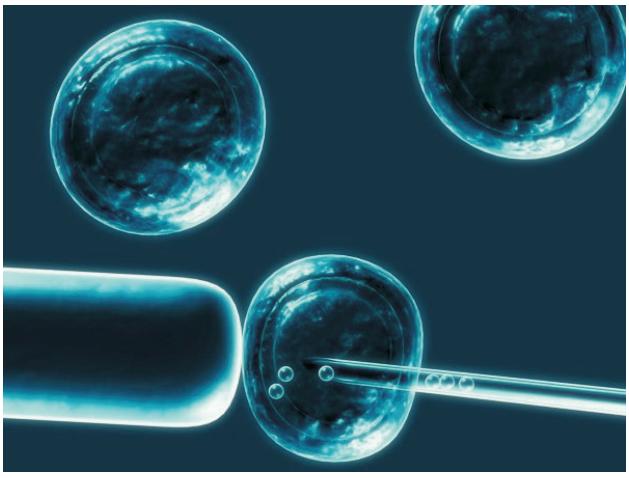

FIGURE 3. Cell selection under a microscope.

Isolation of Animal Cells

All animal cells are significantly larger than microbes. This allows for more direct selection of the cells that are to be cultured. If a sample of animal tissue containing the desired cells is examined under a microscope using micromanipulators, you can collect the desired cell type.

Cells in fluid, such as blood cells, can be separated by centrifugation. A **centrifuge**, a

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machine to spin tubes of cells at high speeds, is used to separate cells by size or weight. Cells taken from different levels are then placed under the microscope for final selection.

Isolation of Plant Cells

Plants can regenerate more easily than animals. In many cases, it is possible to take a portion of a plant and, by placing it in an environment with water and nutrients, get it to grow a mass of undifferentiated tissue. This mass is called **callus** (undifferentiated cells of a plant growing in culture). All the cells have the same genetic information. If a mass of callus is placed in a sterile medium and agitated, the cells will break apart, establishing a cell suspension. A **cell suspension** is a population of the same type of cells growing in a liquid medium.

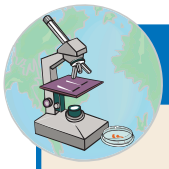
MAINTAINING CELL CULTURES

Once you establish a cell culture, it is important to keep it going. Cell cultures are used to do very important research and produce important products. To maintain continuity in research, the same type of cells must be used at all steps, so the culture used may have to be maintained for years.

Reculturing

One procedure that is very important to all cell cultures is reculturing. **Reculturing** is the act of taking a sample of a culture under sterile conditions and placing it into new sterile media to keep the culture going. With microbial cultures this can be done indefinitely. Animal cells have a limit as to how many times they can divide. The number of times is called the Hayflick limit and is between 40 and 70 times in humans, depending on cell type. In both animals and plants, the Hayflick limit leads to **senescence**, or aging.

Some cell cultures get past this limit by using cells with mutations that make them **immortal**, or able to keep dividing indefinitely. In plants, cells taken from plant organs and



EXPLORING OUR WORLD...

SCIENCE CONNECTION: Cell Culture of Flu Vaccine

Several times in recent years, shortages of flu vaccine have occurred at the start of an outbreak. There are various reasons, but the main problem is that the vaccine is grown in eggs and the process takes about six months to complete.

If an unexpected strain emerges or more vaccine than anticipated is needed, there is no way to change what was started. Alternative methods are needed if we are going to keep ahead of the rapidly changing flu viruses.

One method that is actually in production and testing is using cell cultures to grow the viruses for vaccines. The viruses are grown in mammalian cells rather than chicken eggs. The cells are grown in large bioreactors, and production can take weeks rather than months. The safety and efficacy of the vaccines need to be verified. This method may be what we need to get the upper hand on the flu viruses.

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dedifferentiated become immortal as well. Where an immortal line is not available, some cells are hybridized with cancer cells to make them immortal. Even if cells are immortal, the culture must be regularly recultured to keep the cells from getting crowded or running out of food or to keep cell metabolic waste from building up.

Feeding

Nutrients must be added to cell cultures to feed the cells. This is done regularly between instances of reculturing. With all cell cultures, aseptic techniques are critical at all times. Most aspects of cell culture work are done in sterile-hood environments. This is particularly important in media preparation.

Culture Conditions

Other culture conditions, such as temperature and light requirements, must be addressed. Some cells need to be agitated as they grow. Many animal cell cultures need to adhere to the surface of the container. When these cells are recultured, they must be released from the surface. This can be done mechanically by shaking or gentle scraping, or it can be accomplished by using enzymes.

QUANTIFYING CELL CULTURES

You need to know that your cell culture is healthy. The best way to keep track of the culture is to count the cells periodically. Samples will usually be taken at feeding or reculturing. The difficulty with the counting is the small size of the cells and the enormous numbers in a small volume. Special tools have been developed to aid in this process.

Counting with a Hemacytometer

A **hemacytometer** is a specially marked microscope slide used for counting blood cells. It has a series of grids, and a specified number of squares are counted for each type of blood cell. You can also use it for any other type of cell in solution. Using a hemacytometer is the classic way to count cells in a specific volume of cell culture. The method is fairly low tech and is still used for many preliminary tests.

High-Tech Cell Counting

One of the most common methods of quantifying a cell culture is the use of a Coulter counter. The counter detects changes in charge as cells are drawn through a small aperture. This device allows the counting of blood cells in seconds versus half an hour for a manual hemacytometer count.

Another high-tech method is to stain the cells and then use video capture through your microscope to send a digital photo to a computer that has been programmed to count the cells.

Flow cytometry uses laser light to illuminate the cells as they pass through very small tubes. The light is picked up by detectors placed at various angles. Thousands of cells per second can be scanned.

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STORING CELL CULTURES

When you are not doing research on a particular line, putting the cells in long-term storage is advantageous. Then, they do not have to be recultured on a regular basis. This reduces the work involved as well as the chances for contamination. It is often a good idea for you to store some cells long term as a backup in case the working culture fails or becomes contaminated. Another reason for storing some of your culture is that continually dividing cells are susceptible to random mutations. Most methods of storage involve cold.

Storing Bacterial Cells

Bacterial cultures should not be allowed to grow in an incubator for more than a day or two after colonies arise. They can overgrow and die.

If a culture needs to be stored for a short time, it should be placed in a refrigerator and sealed to prevent drying. It will keep for up to a month without reculturing.

To keep a bacterial culture longer, there are several methods. One simple method is to make a stab tube. You transfer a bacterial colony by stabbing it deeply into a tube of nutrient agar. The tube is sealed and placed in the dark. It may be kept for several months or up to a year. This will work with only some bacteria.

Longer-term storage is possible by mixing the bacteria with an equal volume of sterile glycerol and freezing the culture rapidly in liquid nitrogen or on dry ice. It can then be stored in a freezer. The culture will remain viable for one to two years at –20°C or for a lifetime at –70°C.

Another method that can be used with bacterial, yeast, and fungal cultures is **lyophilization**, or freeze-drying.

Storing Animal Cells

FIGURE 4. Human cell culture.

Animal cell cultures and some plant cell cultures can be stored indefinitely at –196°C in the gas phase over liquid nitrogen. Animal cells are usually prepared for storage by first displacing part of their water with a storage solution, such as dimethyl sulfoxide (DMSO). It is cold (4°C) when added, and after centrifugation, the cells are placed in a –70°C freezer before transfer to liquid nitrogen. Some procedures place them directly into the liquid nitrogen storage. To recover the culture, you warm it quickly in a 37°C water bath and transfer it to sterile culture media.

Storing Plant Cells

Some plant cells have been preserved in liquid nitrogen storage. The plants are often prepared for this storage by cold treating them to stimulate production of natural cold protection

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chemicals. The cells are then sterilely removed and treated with a 10 percent DMSO or 70 percent glycerol solution and placed in liquid nitrogen gas phase storage, as with animal cells.

Summary:

A very important tool in research is the ability to work with one type of cell at a time. It is necessary to isolate the desired cell type and establish a cell culture. Extreme care must be taken through aseptic techniques to ensure the purity of the culture as it is maintained and recultured. To assess the health of the culture and the results of any research treatment, samples of the culture are counted. To preserve samples for comparison and later research, long-term storage under extremely cold conditions is utilized. Cell culturing is a very important research tool. It requires an attention to detail and a large investment in time and materials.

Checking Your Knowledge:

1. Describe how bacterial cells are isolated on a petri dish.
2. How are animal cells isolated for cell culture?
3. What is the purpose of reculturing cell cultures?
4. Why do cell cultures have to be counted on a regular basis?
5. Describe the process for long-term storage of animal cells.

Expanding Your Knowledge:

If you were the head of the U.S. Department of Health and Human Services, would you use egg-based or cell-based vaccines for the next flu vaccine production? Determine the pros and cons of each method, and justify your decision.

Web Links:

American Type Culture Collection Home Page
http://www.atcc.org

Information on Flu Vaccine Production in Eggs and Cell Culture
http://www.wikipedia.org/wiki/influenza_vaccine

GlaxoSmithKline Background on Cell Culture Vaccine Production
http://www.gsk.com/media/flu/tissue_backgrounder.pdf

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