

Animal Cell Culture

Cell culture refers to the process by which cells are grown in a controlled artificial environment. Cells can be maintained in vitro outside of their original body by this process which is quite simple compared to organ and tissue culture.

For animal cell culture the cells are taken from the organ of an experimental animal. The cells may be removed directly or by mechanical or enzymatic action. The cells can also be obtained by previously made cell line or cell strain. Examples of cells used to culture are **fibroblast, lymphocytes, cells from cardiac and skeletal tissues, cells from liver, breast, skin, and kidney and different types of tumor cells.**

Types of animal cell culture

A. Primary cell culture

- This is the cell culture obtained straight from the cells of a host tissue.
- The cells dissociated from the parental tissue are grown on a suitable container and the culture thus obtained is called primary cell culture.
- Such culture comprises mostly heterogeneous cells and most of the cells divide only for a limited time. However, these cells are much similar to their parents.
- Depending on their origin, primary cells grow either as **an adherent monolayer** or **in a suspension**.

Adherent cells

- ✓ These cells are anchorage dependent and propagate as a monolayer.
- ✓ These cells need to be attached to a solid or semi-solid substrate for proliferation. Fibroblasts and epithelial cells are of such types.

Suspension cells

- Suspension cells do not attach to the surface of the culture vessels.
- These cells are also called anchorage independent or non-adherent cells which can be grown floating in the culture medium.
- Hematopoietic stem cells (derived from blood, spleen and bone marrow) and tumor cells can be grown in suspension.

B. Secondary cell culture and cell line

When a primary culture is sub-cultured, it is known as secondary culture or cell line or sub-clone. The process involves removing the growth media and disassociating the adhered cells (usually enzymatically).

On the basis of the life span of culture, the cell lines are categorized into two types:

➤ Finite cell lines

- ✚ The cell lines which go through a limited number of cell division having a limited life span are known as finite cell lines.
- ✚ The cells passage several times and then lose their ability to proliferate, which is a genetically determined event known as **senescence**. Cell lines derived from primary cultures of normal cells are finite cell lines.

➤ **Continuous cell lines**

- When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line.
- Such transformation/mutation can occur spontaneously or can be chemically or virally induced or from the establishment of cell cultures from malignant tissue.

Common cell lines

Human cell lines:

1. MCF-7 (breast cancer)
2. HL 60 (Leukemia)
3. HeLa (Human cervical cancer cells)

Primates cell lines: Vero (African green monkey kidney epithelial cells)

Growth Requirements

The culture media used for cell cultures are generally quite complex, and culture condition widely varies for each cell type. However, media generally include **amino acids** (source of nitrogen), **vitamins** (cofactors), **salts** (maintain osmotic pressure), **glucose** (source of energy , carbon,) **a bicarbonate buffer system** (maintains a pH between 7.2 and 7.4), **growth factors and hormones** (growth stimulators), **O₂** and **CO₂**. To obtain best growth, addition of a small amount of **blood serum** is usually necessary, and several **antibiotics**, like penicillin and streptomycin are added to prevent bacterial contamination.

Temperature varies on the type of host cell. Most mammalian cells are maintained at 37°C for optimal growth, while cells derived from cold- blooded animals tolerate a wider temperature range (i.e. 15°C to 26°C).

Process to obtain primary cell culture

Primary cell cultures are prepared from fresh tissues.

- ✓ Pieces of tissues from the organ are removed aseptically; which are usually minced with a sharp sterile razor and dissociated by proteolytic enzymes (such as trypsin) that break apart the intercellular cement.
- ✓ The obtained cell suspension is then washed with a physiological buffer (to remove the proteolytic enzymes used).
- ✓ The cell suspension is spread out on the bottom of a flat surface, such as a bottle or a Petri dish.
- ✓ This thin layer of cells adhering to the glass or plastic dish is overlaid with a suitable culture medium and is incubated at a suitable temperature.

Applications of Cell Line

A. Vaccines Production

One of the most important uses of cell culture is in research and production of vaccines. The ability to grow large amounts of virus in cell culture eventually led to the creation of the polio vaccine, and cells are still used today on a large scale to produce vaccines for many other diseases, like rabies, chicken pox, hepatitis B, and measles.

B. Virus cultivation and study

Cell culture is widely used for the propagation of viruses as it is convenient, economic, easy to handle compared to other animals.

C. Cellular and molecular biology

Cell culture is one of the major tools used in cellular and molecular biology, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging), the

effects of different toxic compounds on the cells, and mutagenesis and carcinogenesis.

D. In Cancer Research

Normal cells can be transformed into cancer cells by methods including radiation, chemicals and viruses.

E. Gene therapy

Cells having a functional gene can be replaced to cells which are having non-functional gene, and for which the cell culture technique is used.

F. Immunological studies

Cell culture techniques are used to know the working of various immune cells, cytokines, lymphoid cells, and interaction between disease causing agent and host cell.

G. Others

Cell lines are also used in in-vitro fertilization (IVF) technology, recombinant protein and drug selection and improvement.

