

# UNIT-V

## Cell culture procedure



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# Animal cell culture

- Cell culture is the process by which human, animal, or insect cells are grown in a favorable artificial environment. The cells may be derived from **multi-cellular eukaryotes**, already established cell lines or established cell strains.
- Animal cell culture is now one of the major tools used in the **life sciences in areas of research** that have a **potential for economic value and commercialization.**

- The development of basic culture media has enabled scientists to work with a wide variety of cells under **controlled conditions**; this has played an important role in;
  - **advancing our understanding of cell growth**
  - **differentiation, identification of growth factors,**
  - **understanding of mechanisms underlying the normal functions** of various cell types.
- New technologies have also been applied to investigate **high cell density bioreactor** and culture conditions.

## **Cell culture is of three types:**

(1) **Precursor cell culture**, which is undifferentiated cells committed to differentiate;

(2) **Differentiated cell culture**, which is completely differentiated cells that have lost the capacity to further differentiate; and

(3) **Stem cell culture**, which is undifferentiated cells that go on to develop into any type of cell.

- Cells with a defined cell type and characteristics are selected from a culture by cloning or by other methods; this cell line becomes a cell strain.

### ***Monolayer cultures***

- The monolayer culture is **an anchorage-dependent culture** of usually one cell in thickness with a continuous layer of cells at the bottom of the culture vessel.

### ***Suspension cultures***

- Some of the cells are **non adhesive** and can be mechanically kept in suspension, unlike most cells that grow as mono-layers (e.g., cells of leukemia). This offers numerous advantages in the propagation of cells.

# Advantages of animal cell culture

**1. Physiochemical and physiological condition:** Role and effect of pH, temperature,  $O_2/CO_2$  concentration, and osmotic pressure of the culture media can be altered to study their effects on the cell culture.

**2. Metabolism of cell:** To study cell metabolism and investigate the physiology and biochemistry of cells.

**3. Cytotoxic assay:** Effect of various compounds or drugs on specific cell types such as liver cells can be studied.

**4. Homogenous cultures:** These cultures help study the biology and origin of the cells.

# General procedure for cell culture

- 1) First of all we have to give UV radiation to culture hood for sterilisation.
- 2) Media which was kept in 4°C should be remain on room temperature before start.
- 3) Clean the Hood with 70% ethyl alcohol.
- 4) Then keep all necessary things in culture hood after rinsing them with alcohol.
- 5) Discard should be there in hood to discard the spent media of culture flask.
- 6) Check the confluency of cells first under microscope. Cells should be healthy.
- 7) Never split the cells which are not confluent. Otherwise it will stress to the cells. Cells are very fragile and sensitive so handle very gently and carefully.
- 8) Always clean your hands with 70% ethyl alcohol.
- 9) Trypsin/EDTA which is required for cell splitting should be pre-warmed at 37 °C.

## Procedure for splitting/passaging of cell line from established primary cell line (Like Vero cell line which is an adherent cell line)

- 1) Take the confluent flask and remove all the spent media using serological pipette.
- 2) Wash the monolayer of cells once with Phosphate buffer saline.
- 3) Then take 1 mL of Trypsin/EDTA solution and let it cover the monolayer completely.
- 4) Remove some trypsin solution.
- 5) Keep the flask at 37°C in BOD incubator for approx. one and half minutes.
- 6) Tap gently on the side of flask so that cells can dislodge.
- 7) Add about 2-3 mL of 10% complete DMEM media to flush off the cells that have got dislodged.
- 8) Centrifuge the tube containing the cells at 1500 rpm for 5 minutes.
- 9) Cell pellet will form.
- 10) Now dissolve the pellet in 2 ml fresh DEMEM media.
- 11) Count the cells by hemocytometer and take the cells accordingly into the new flask to continue the cell line.
- 12) Check the flask after 24 hour for its quality and any contamination.
- 13) Split this flask at alternate day to maintain the cell line for long time (i.e. upto 30 passages).



# How freezing of cells is done in Liquid nitrogen

- After trypsinisation of Confluent culture flask , forms pellet and dissolve in DMEM media(as mentioned in previous slide).
- Count the cell to get an idea about cell density.
- Then add 2-3 million cells per cryovial on freezing media.
- Freezing media is basically DMEM media with 10% FBS and 10% DMSO). DMSO is dimethyl sulphoxide which is a cryoprotectant and prevent ice crystal formation.
- Keep the cryovial in  $-80^{\circ}\text{C}$  and then next day in liquid nitrogen.
- In this way our cell line remains with us for a very long time.

# Source of contamination in cell culture

- Contamination of cell line means something which is undesirable in the culture system but comes unknowingly.
- Contamination makes our culture media present in cell culture flask fuzzy. Normally it should be clear.
- Two reasons may be possible for contamination either
  - 1. Chemical contamination
  - 2. Biological contamination.
- **Chemical contamination includes:-**
  - ✓ DMEM media
  - ✓ Phosphate buffer
  - ✓ Trypsin/EDTA solution
  - ✓ Incubator
- **Biological contamination includes:-**
  - ✓ Bacteria
  - ✓ Fungi
  - ✓ Mycoplasma (generally observed)
  - ✓ Cross contamination with other cell line (i.e. When two cell lines are split back to back without giving any UV-radiation, then chances are more).

# Growth of animal cells in culture

- The cells in the culture show a characteristic growth pattern, **lag phase, exponential or log phase, followed by a plateau phase.**
- The **population doubling time** of the cells can be calculated during the **log phase** and **plateau phase.**
- This is **critical** and can be used **to quantify the response of the cells to different culture conditions for changes in nutrient concentration and effects of hormonal or toxic components.**
- The **population doubling time** describes the **cell division rates within the culture** and is **influenced by non-growing and dying cells.**

# Phases of the growth cycle

- The population doubling time, lag time, and saturation density of a particular cell line can be established and characterized for a particular cell type.
- A growth curve consists of a normal culture and can be divided into a lag phase, log phase, and plateau phase.

## Lag phase

- This is the initial growth phase of the subculture and re-seeding during which the cell population takes time to recover.
- The cell number remains relatively constant prior to rapid growth.
- During this phase, the cell replaces elements of the **glycocalyx lost during trypsinization**, attaches to the substrate, and spreads out.
- During the spreading process, the **cytoskeleton reappears**; its reappearance is probably an integral part of the process.

## Log phase

- This is a period of **exponential increase in cell number and growth of the cell population** due to **continuous division**.
- The **length** of the log phase **depends on the initial seeding density**, the **growth rate of the cells**, and the **density at which cell proliferation is inhibited** .
- This phase represents the **most reproducible** form of the culture as **the growth fraction and viability is high** (usually 90%–100%), and the population is at its most uniform.
- However, the cell culture may not be synchronized, and the cells can be randomly distributed in the cell cycle.

## Plateau phase

- The culture becomes **confluent at the end of the log phase** as **growth rates** during this phase are **reduced**, and **cell proliferation can cease** in some cases due to **exhaustion**.
- The cells are in contact with surrounding cells, and the growth surface is occupied.
- At this stage, the culture enters the stationary phase and the growth fraction falls to between 0% and 10%.
- Also, the constitution and charge of the cell surface may be changed, and there may be a relative increase in the synthesis of specialized versus structural proteins.

# Primary, established and transformed cell cultures

## Primary cell culture

- These cells are obtained **directly from tissues and organs** by **mechanical or chemical disintegration** or by **enzymatic digestion**.
- These cells are induced to grow in suitable glass or **plastic containers** with **complex media**.
- These cultures usually have a **low growth rate** and are **heterogeneous**; however, they are **still preferred over cell lines** as these are **more representative of the cell types** in the tissues from which they are derived.



The morphological structure of cells in culture is of various types:

- **(1) Epithelium type**, which are polygonal in shape and appear flattened as they are attached to a substrate and form a continuous thin layer (i.e., monolayer on solid surfaces);
- **(2) Epitheloid type**, which have a round outline and do not form sheets like epithelial cells and do not attach to the substrate;
- **(3) Fibroblast type**, which are angular in shape and elongated and form an open network of cells rather than tightly packed cells, are bipolar or multipolar, and attach to the substrate; and
- **(4) Connective tissue type**, which are derived from fibrous tissue, cartilage, and bone, and are characterized by a large amount of fibrous and amorphous extracellular materials.

- Depending on the kind of cells in culture, the primary cell culture can also be divided into two types.

### **Anchorage-dependent/adherent cells**

- These cells require a **stable nontoxic and biologically inert surface for attachment and growth** and are **difficult to grow as cell suspensions**. Mouse fibroblast STO cells are anchorage cells.

### **Anchorage-independent/suspension cells**

- These cells **do not require a solid surface for attachment or growth**.
- Cells **can be grown continuously in liquid media**. The source of cells is the **governing factor** for suspension cells. **Blood cells are vascular in nature** and are **suspended in plasma** and these cells can be very easily established in suspension cultures.

## Advantages and disadvantages of primary cell culture

- These cultures represent the best experimental models for in vivo studies.
- They share the same karyotype (complete set of chromosome) as the parent and express characteristics that are not seen in cultured cells.
- However, they are difficult to obtain and have limited life spans. **Potential contamination** by viruses and bacteria is also a major disadvantage.

## **Disadvantages of animal cell culture**

- 1. Expenditure and expertise:** This is a specialized technique that requires aseptic conditions, trained personnel, and costly equipment.
- 2. Dedifferentiation:** Cell characteristics can change after a period of continuous growth of cells in cultures, leading to differentiated properties compared to the original strain.
- 3. Low amount of product:** The minimum amount of mAB and recombinant protein produced followed by downstream processing for extracting pure products increases expenses tremendously.

**4. Contamination:** Mycoplasma and viral infection are difficult to detect and are highly contagious.

**5. Instability:** Aneuploidy (abnormal chromosome) chromosomal constitution in continuous cell lines leads to instability. In addition, this system cannot replace the complex live animal for testing the response of chemicals or the impact of vaccines or toxins.

**5. Valuable biological data from large-scale cell cultures:**

Specific proteins can be synthesized in large quantities from genetically modified cells in large-scale cultures.

**6. Consistency of results:** Reproducibility of the results that can be obtained by the use of a single type/clonal population.

**7. Identification of cell type:** Specific cell types can be detected by the presence of markers such as molecules or by karyotyping.

**8. Ethics:** Ethical, moral, and legal questions for utilizing animals in experiments can be avoided.

# Application of cell cultures in pharmaceutical industry and research

Recently cell lines have transformed scientific study and are used for several purposes, such as:

- Vaccine production.
- Examining drug metabolism.
- Cytotoxicity.
- Antibody production.
- Investigating gene function.
- Development of artificial tissues (e.g. artificial skin).
- Production of biological compounds (e.g. therapeutic proteins)