

# UNIT-V

## Animal cell culture



**CENTURION**  
**UNIVERSITY**  
*Shaping Lives...  
Empowering Communities!*

**RASMITA JENA**  
**ASSISTANT PROFESSOR**  
**SoPLS, CUTM, BBSR**

# 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.

- Animal cell culture has found use in diverse areas, from basic to advanced research. It has provided a **model system for various research efforts**:
  1. The study of basic **cell biology, cell cycle mechanisms**, specialized cell function, **cell–cell** and **cell–matrix interactions**.
  2. **Toxicity testing** to study the effects of new drugs.
  3. **Gene therapy** for replacing nonfunctional genes with functional gene-carrying cells.
  4. The **characterization of cancer cells**, the role of various chemicals, viruses, and radiation in cancer cells.
  5. **Production of vaccines, mABs, and pharmaceutical drugs**.
  6. **Production of viruses** for use in **vaccine production** (e.g., chicken pox, polio, rabies, hepatitis B, and measles).

- 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.

# Cell lines generally used in lab



Cell name	Tissue	Phenotype	Application
Sf9 ( <i>Spodoptera frugiperda</i> )	Insect ovary	Suspension	Protein expression, virus production, transient transfection
BHK-21	Hamster kidney	Normally grown adherently but sometimes adapted to suspension culture	Protein expression, virus production, transient transfection
Vero	African monkey kidney	Adherent	Virus isolation, Antiviral drug testing, Plaque Reduction Neutralization Test (PRNT)
C6/36	<i>Aedes aegypti</i> larva	Adherent	Preferentially used for replication of Dengue virus to high titer.

# Requirement of cell culture

- A substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals).
- Growth factors (Fetal Bovine Serum)
- Hormones
- Gases ( $O_2$ ,  $CO_2$ )
- A regulated physico-chemical environment (pH, osmotic pressure, temperature).
- Bio safety cabinet/Laminar hood/ BOD
- Serological sterile pipette,
- Trypsin/EDTA solution
- Phosphate buffer saline
- T-25/T-75 culture flask.
- Penicillin/streptomycin solution (required to prevent bacterial contamination).

For enhancing growth and proliferation of cells

# Animal cell culture media

Category	Definition	Type	Example
Natural media	Consisting of natural biological substances, such as plasma, serum, and embryo extract	Coagulant or clots	Plasma separated from heparinized blood, serum, and fibrinogen
		Tissue extracts	Extracts of chicken embryos, liver, and spleen and bone marrow extract
		Biological fluids	Plasma, serum, lymph, amniotic fluid, and pleural fluid
Synthetic media	Composed of a basal medium and supplements, such as serum, growth factors, and hormones	Serum-containing media	Human, bovine, equine, or other serum is used as a supplement
		Serum-free media	Crude protein fractions, such as bovine serum albumin or $\alpha$ - or $\beta$ -globulin, are used as supplements
		Xeno-free media	Human-source components, such as human serum albumin, are used as supplements but animal components are not allowed as supplements
		Protein-free media	Undefined components, such as peptide fractions (protein hydrolysates) are used as supplements
		Chemically defined media	Undefined components, such as crude protein fractions, hydrolysates, and tissue extracts, are not appropriate as supplements, but highly purified components, such as recombinant proteins are appropriate supplements



## How are cell cultures obtained?

There are three methods commonly used to initiate a culture from animals.

### 1. Organ culture

- Whole organs from embryos or partial adult organs are used to initiate organ culture in vitro.
- These cells in the organ culture maintain their differentiated character, their functional activity, and also retain their in vivo architecture.
- They do not grow rapidly, and cell proliferation is limited to the periphery of the explant.

- As these cultures cannot be propagated for long periods, a fresh explanation is required for every experiment that leads to inter experimental variation in terms of reproducibility and homogeneity.
- Organ culture is useful for
  - **studying functional properties of cells (production of hormones)**
  - **examining the effects of external agents (such as drugs and other micro or macro molecules)**
  - **products on other organs that are anatomically placed apart in vivo.**

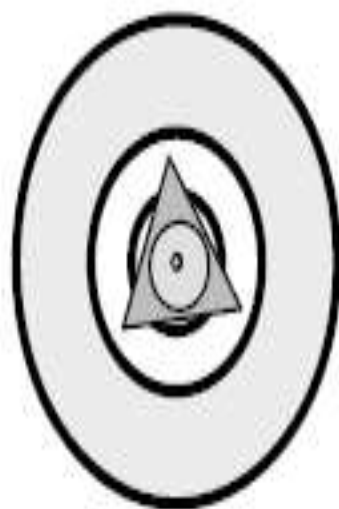
## 2. Primary explant culture

- Fragments excised from animal tissue may be maintained in a number of different ways.
- The tissue adheres to the surface aided by an extracellular matrix (ECM) constituent, such as collagen or a plasma clot, and it can even happen spontaneously.
- This gives rise to **cells migrating from the periphery of the explant**. This culture is known as a **primary explant**, and **migrating cells are known as outgrowth**.
- This has been used to analyze **the growth characteristics of cancer cells** in comparison to their normal counterparts, especially with reference to **altered growth patterns and cell morphology**.

### 3. Cell culture

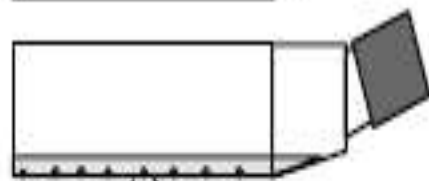
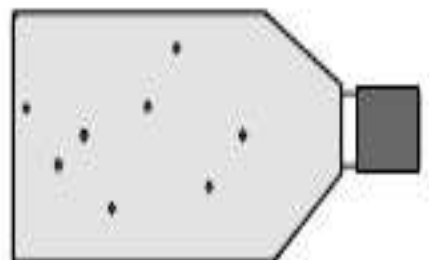
- This is the most commonly used method of tissue culture and is generated by collecting the cells growing out of explants or dispersed cell suspensions (floating free in culture medium).
- Cells obtained either by enzymatic treatment or by mechanical means are cultured as adherent monolayers on solid substrate.

# ORGAN CULTURE



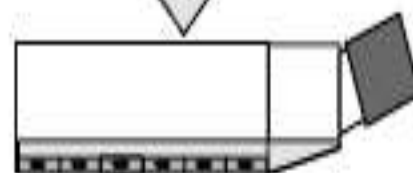
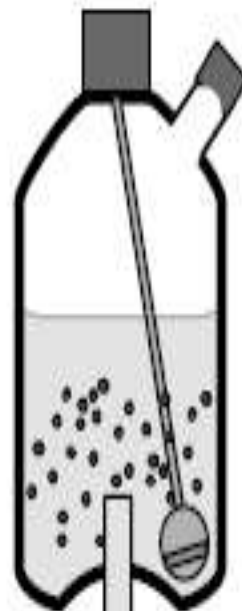
Tissue at gas-liquid interface; histological structure maintained

# EXPLANT CULTURE



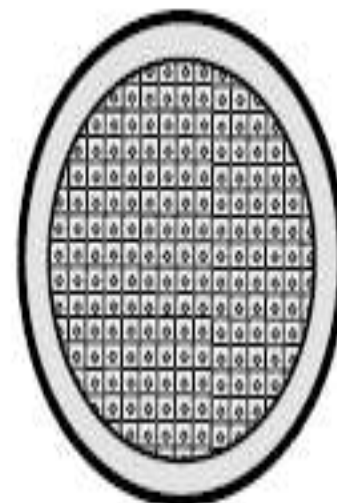
Tissue at solid-liquid interface; cells migrate to form outgrowth

# DISSOCIATED CELL CULTURE

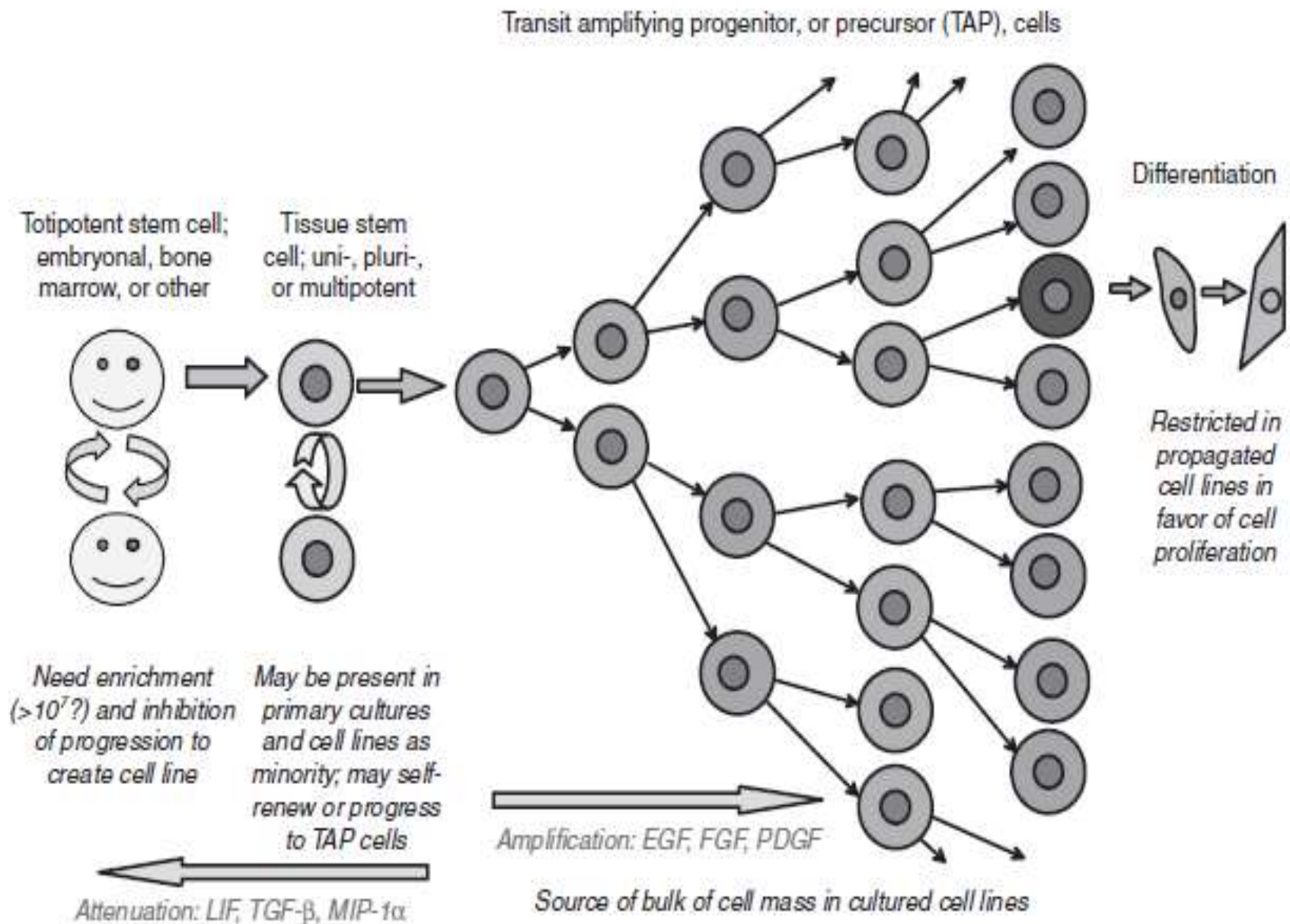


Disaggregated tissue; cells form monolayer at solid-liquid interface

# ORGANOTYPIC CULTURE



Different cells co-cultured with or without matrix; organotypic structure recreated



## Major equipments required for cell culture



Cell culture incubator

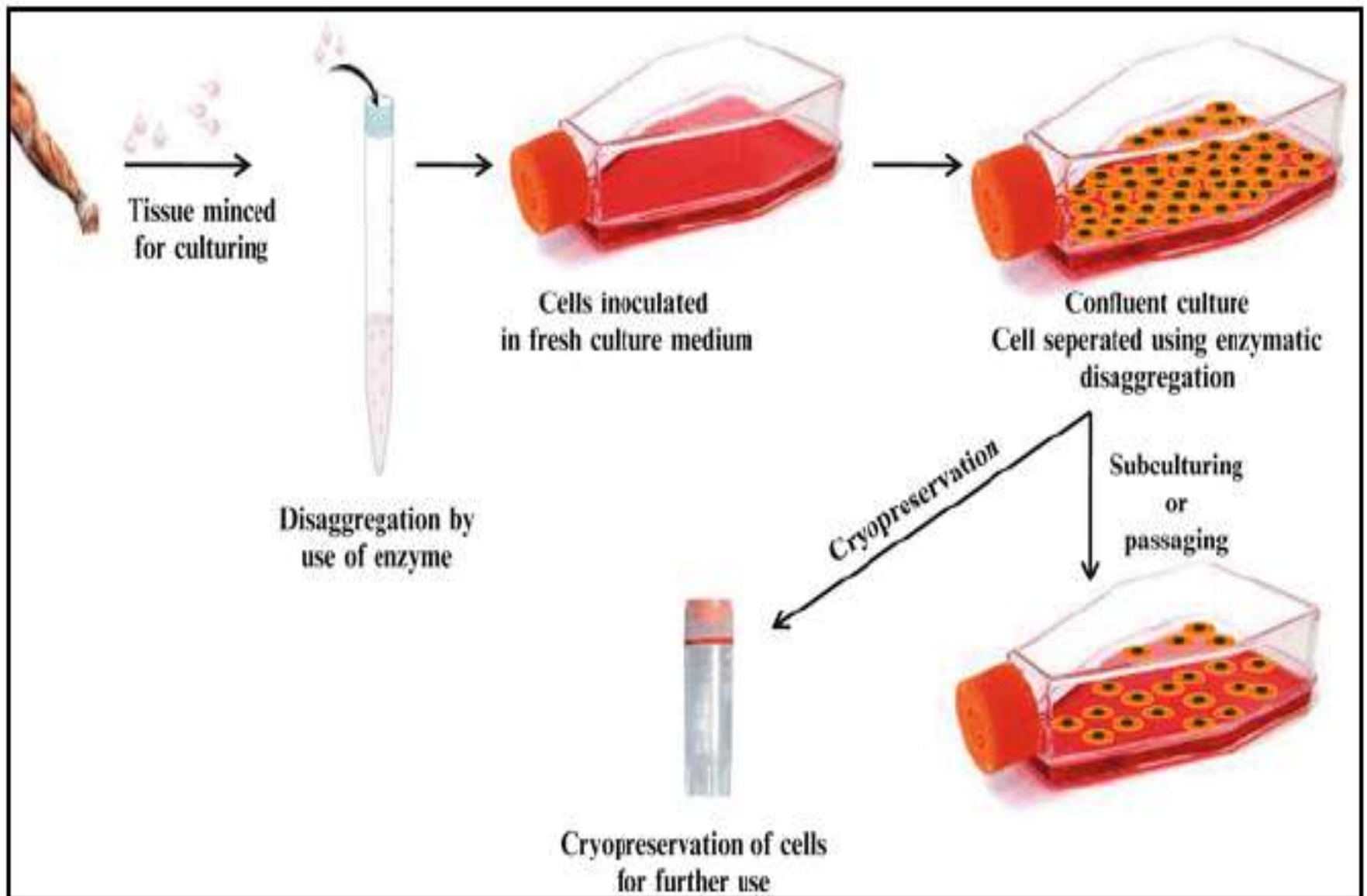


Bio Safety cabinet



Sub-culturing of cells on confluency

# Overview of how cell culture is performed in culture lab





## **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.