Unit-5

Cell culture-

Cell culture is the process of growing cells outside of their natural environment, usually in a lab dish or flask.

Types of Cell Culture:-

Primary Culture: cells taken directly from living tissue

Cell Lines: cells that can grow continuously in the lab

Suspension Culture: cells grown floating in liquid

Adherent Culture: cells grown attached to a surface

Why Do We Use Cell Culture?

To study cell behavior
To test drugs and treatments
To produce biological products like vaccines and antibodies
For research in genetics, cancer, and more

Basic Equipment in Cell Culture Lab:-

1. Biological Safety Cabinet (Laminar Flow Hood)
Provides sterile environment to handle cells safely.

2. CO2 Incubator
Maintains proper temperature, humidity, and CO2 levels for cell growth.

3. Microscope
To observe cells and check their health and growth.

4. Centrifuge
Separates cells from liquids or other components.

5. Water Bath
Keeps media and reagents warm at a controlled temperature.

6. Autoclave
Sterilizes equipment and culture media by using high-pressure steam.

7. Pipettes and Pipette Tips
For transferring precise volumes of liquids.

8. Culture Vessels
Petri dishes, flasks, and plates where cells are grown.

9. Refrigerator and Freezer
Store media, reagents, and cell samples.

10. Vortex Mixer-

Mixes solutions quickly.

Cell Culture Media

Cell culture media is a specially formulated liquid or gel that provides all the nutrients and environment cells need to grow and survive outside the body.

Purpose of Cell Culture Media-

Supplies essential nutrients
Maintains the correct pH and osmotic balance
Provides growth factors and hormones
Removes waste products from cells
Supports cell attachment and growth

Types of Cell Culture Media

1. Basal Media

Contains basic nutrients: amino acids, vitamins, salts, glucose, and buffering agents.
Examples: DMEM (Dulbecco's Modified Eagle Medium), RPMI 1640, MEM (Minimum Essential Medium).

2. Complete Media

Basal media + serum (usually fetal bovine serum, FBS) and additional growth factors, hormones, or antibiotics.

3. Serum-Free Media

Formulated without serum, contains defined growth factors and supplements.
Used to avoid variability and for specific cell types.

4. Specialized Media

Designed for specific cells or applications, like stem cell media or media for insect cells.

Components of Cell Culture Media

Component	Role
Amino acids	Building blocks for proteins
Glucose	Main energy source for cells
Vitamins	Help with cell metabolism and growth
Salts (ions)	Maintain osmotic balance and pH
Buffers	Keep pH stable (e.g., bicarbonate, HEPES)
Serum	Provides growth factors, hormones, attachment factors, and proteins
Growth factors	Stimulate cell growth and division
Antibiotics	Prevent bacterial contamination

Types of Cell Culture

1. Primary Cell Culture

Cells are taken directly from living tissues (e.g., liver, skin).

These cells are fresh and behave like they do inside the body.

Limitations: They don't divide forever — they die after a few divisions.

2. Secondary Cell Culture (Subculture)

When primary cells grow too many, they are transferred to new vessels.

This process is called passaging.

Cells continue to grow but may lose some original features over time.

3. Cell Lines

Cells that can grow continuously for many generations.

Often come from cancer cells or are genetically modified.

Example: HeLa cells (from cervical cancer).

Can be:

Finite cell lines – grow for a limited number of divisions

Continuous (immortal) cell lines – grow forever

4. Adherent Cell Culture

Cells grow attached to the surface of the dish or flask.

Common for cells from solid tissues (e.g., skin, kidney).

Needs a flat surface to grow.

5. Suspension Cell Culture

Cells grow freely floating in the medium.

Common for blood cells or immune cells.

Grown in shaking flasks or bioreactors.

6. 3D Cell Culture

Cells grow in a three-dimensional structure (like in the body).

Gives a more realistic environment.

Used in cancer, stem cell, and drug research.

General Procedure for cell culture

isolation of cell , sub culture-

1. Cell Isolation (from tissue or blood)

Purpose: To get live cells from tissues or organs for culture.

Steps:

1. Collect Tissue or Blood Sample

From animal, plant, or human source using sterile methods.

2. Wash the Tissue

Use sterile buffer (like PBS) to remove blood or dirt.

3. Cut and Mince

Chop tissue into small pieces using sterile scissors.

4. Digest with Enzymes

Use enzymes like trypsin or collagenase to break tissue into single cells.

5. Filter and Centrifuge-
Filter to remove debris; centrifuge to collect cells.

6. Resuspend in Culture Medium
Add suitable growth medium to cells.

7. Seed Cells in Culture Dish
Place cells in a flask or dish and incubate at 37°C with 5% CO_2.

2. Subculture (Passaging)

Purpose: To transfer growing cells to a new vessel to prevent overcrowding.

Steps:

1. Check Confluency
Cells should cover 70-90% of the surface before passaging.

2. Remove Old Media
Use sterile pipette to discard used culture media.

3. Rinse with PBS (without calcium/magnesium)
Wash away dead cells and residues.

4. Add Trypsin (or enzyme)
Detaches cells from surface.

5. Incubate Briefly
Wait 2–5 minutes until cells round up and detach.

6. Neutralize Trypsin with Fresh Media
Add complete medium with serum to stop enzyme activity.

7. Centrifuge and Resuspend Cells
Pellet cells, discard supernatant, and add fresh media.

8. Transfer to New Flask
Split into new culture vessels and return to incubator.

Cryopreservation (Freezing Cells for Long-Term Storage)

Purpose: To store cells at ultra-low temperatures for future use.

Steps:

1. Harvest Healthy Cells

Use trypsin to detach and collect cells at log phase growth.

2. Centrifuge and Resuspend

Pellet cells and resuspend in freezing medium: Usually 90% FBS + 10% DMSO (cryoprotectant).

3. Aliquot into Cryovials

Add 1–2 mL of cell suspension into sterile cryovials.

4. Slow Cooling

Freeze gradually to -80°C (e.g., using a freezing container or step-wise freezer).

5. Transfer to Liquid Nitrogen

Store at -196°C in liquid nitrogen for long-term preservation.

Characterization of Cells & their applications

Characterization means identifying and confirming the type, quality, and behavior of cultured cells.

Common Methods to Characterize Cells:

Method	Purpose
Microscopy	Observe cell shape, growth, and health.
Viability Tests	Check if cells are alive (e.g., Trypan Blue).
Growth Curve	Measure cell division over time.
Karyotyping	Check chromosome number and structure.
Immunostaining	Use antibodies to detect specific proteins.
Flow Cytometry	Analyze cell size, surface markers, or DNA.
PCR/RT-PCR	Detect gene expression of cell-type markers.
Mycoplasma Testing	Ensure there is no contamination.

Applications of Cell Culture

Cell culture is widely used in biology, medicine, and industry.

Main Applications:

1. Drug Testing and Development

Screen and test drugs on human cells before using animals or humans.

2. Cancer Research

Study cancer cell behavior, mutations, and treatment responses.

3. Vaccine Production

Grow viruses in cell lines to produce vaccines (e.g., polio, COVID-19).

4. Tissue Engineering

Grow cells to make artificial skin, organs, or tissues.

5. Stem Cell Research
Study stem cells for regenerative medicine and development.

6. Genetic Engineering
Modify genes in cultured cells to study their functions or create therapeutic proteins.

7. Toxicology Testing
Check whether chemicals or cosmetics are toxic to cells (alternative to animal testing).

8. Monoclonal Antibody Production
Use hybridoma cells to produce antibodies for diagnostics and therapy.

Principle and applications of cell viability assay , glucose uptake , calcium influx assay

1. Cell Viability Assay-

A test used to measure the number of living (viable) cells in a sample.

Procedure:

1. Seed Cells in 96-Well Plate

Add cells (e.g., 5,000–10,000 cells per well) in complete medium.

Incubate at 37°C, 5% CO_2 overnight to let them attach.

2. Treat Cells (Optional)

Add test compounds (drugs or chemicals) to the wells.

Incubate for desired time (e.g., 24–48 hours).

3. Add MTT Solution

Add 10–20 µL of MTT (5 mg/mL) to each well.

Incubate for 2–4 hours.

Live cells will convert MTT to purple formazan crystals.

4. Remove Medium

Carefully remove the media (containing MTT that wasn't used).

Be gentle to not disturb the crystals.

5. Add DMSO

Add 100 µL DMSO to each well to dissolve the purple crystals.

6. Measure Absorbance

Use a microplate reader to measure absorbance at 570 nm.

7. Interpret Results

More purple color = more live cells

Compare treated vs. control to assess cell viability.

DEPTH OF BIOLOGY

Principle:

Measures the number of living cells in a sample. Based on metabolic activity, membrane integrity, or enzyme activity.

Live cells convert certain dyes or chemicals into detectable signals (color, fluorescence).

Applications:

Testing drug toxicity
Determining cell health
Measuring effects of treatments
Screening compounds for cell survival

2. Glucose uptake Assay-

A test that measures how much glucose a cell takes in from its surroundings.

Principle:

Measures how much glucose cells take in from the culture medium.
Uses radiolabeled glucose or fluorescent glucose analogs (like 2-NBDG).
Indicates metabolic activity and insulin sensitivity.

Applications:

Study of diabetes and insulin function
Understanding cancer metabolism
Drug screening for metabolic diseases
Measuring energy demand in cells

Procedure-

1. Prepare Cells

Seed cells in a 96-well plate and grow until 70-80% confluent.

2. Starve Cells

Wash cells and incubate in glucose-free medium for 1 hour.

This increases glucose uptake sensitivity.

3. Add 2-NBDG

Add 2-NBDG (e.g., 100 μM) to the wells.

Incubate for 30–60 minutes at 37°C.

4. Wash Cells

Remove the solution and wash cells gently with PBS to remove excess 2-NBDG.

5. Measure Fluorescence

Use a plate reader or microscope.

Excitation: ~465 nm, Emission: ~540 nm.

6. Analyze Data

Compare treated vs. control wells.

More fluorescence = more glucose uptake.

3. Calcium influx assay-

A test that measures the movement of calcium ions (Ca^{2+}) into or within cells.

Principle:

When calcium enters the cell, it binds to fluorescent dyes that glow more under a microscope or reader.

Applications:

Studying cell signaling

Understanding nerve and muscle function

Testing drugs that affect receptors and channels

Procedure:

1. Prepare Cells

Grow cells in a suitable plate or coverslip until they reach the desired density.

2. Load Dye

Add Fluo-4 AM dye (e.g., 2–5 μM) in loading buffer.

Incubate cells at 37°C for 30–45 minutes in the dark.

3. Wash Cells

Wash 2–3 times with calcium-containing buffer to remove extra dye.

4. Equilibrate Cells

Let cells sit for 15–20 minutes to stabilize before measurement.

5. Stimulate Cells

Add the drug or stimulus that triggers calcium entry (e.g., ATP, ionophore).

6. Measure Fluorescence-

Immediately measure calcium influx using a fluorescence microscope or plate reader.
Excitation: ~494 nm, Emission: ~516 nm.

Principle & Applications of Flow Cytometry

Flow cytometry is a lab technique used to count, sort, and study cells one by one as they flow through a laser beam.

Flow cytometry is a powerful technique used in biology and medicine to analyze cells quickly and accurately. It helps scientists study thousands of cells per second, giving information about cell size, shape, and specific markers using light and fluorescence.

This method is widely used in research, clinical diagnosis, and drug development.

Flow Cytometry – Procedure:

1. Collect Cells

Take cells from blood, tissue, or culture.

2. Stain Cells

Add fluorescent antibodies or dyes that bind to specific cell parts.

3. Wash Cells

Remove extra dye by washing with buffer.

4. Run Sample in Flow Cytometer

Put cells into the machine; they pass through a laser one by one.

5. Detect Signals

The machine records light scatter and fluorescence for each cell.

6. Analyze Data

Use computer software to identify, count, or sort the cells

DEPTH OF BIOLOGY

Principle:

Cells are placed in a liquid and passed through a laser light.
The light is scattered and can also make fluorescent dyes on the cells glow.
A machine detects these signals to tell:
Cell size
Shape
What's on or inside the cell

Applications:

Counting immune cells (like T cells, B cells)
Detecting cancer cells in blood
Finding dead or live cells
Studying cell cycle
Checking for infections
Sorting specific cells