Department of Medical Laboratories Techniques
Human genetic
Lab.11: cell culture
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- **Cell culture** is a technique which involves isolation of cells from animal/plant body i.e. from their natural environment (*in vivo*) and practicing to grow isolated cells in cell specific media in plastic flask or petri dish in a controlled environmental artificial condition (*in vitro*).
- Cell culture means to keep cells alive and grow in vitro condition in a nutritive media which are widely used for research and diagnosis of different pathogens and to understand the function and mechanism of operation of many cells.

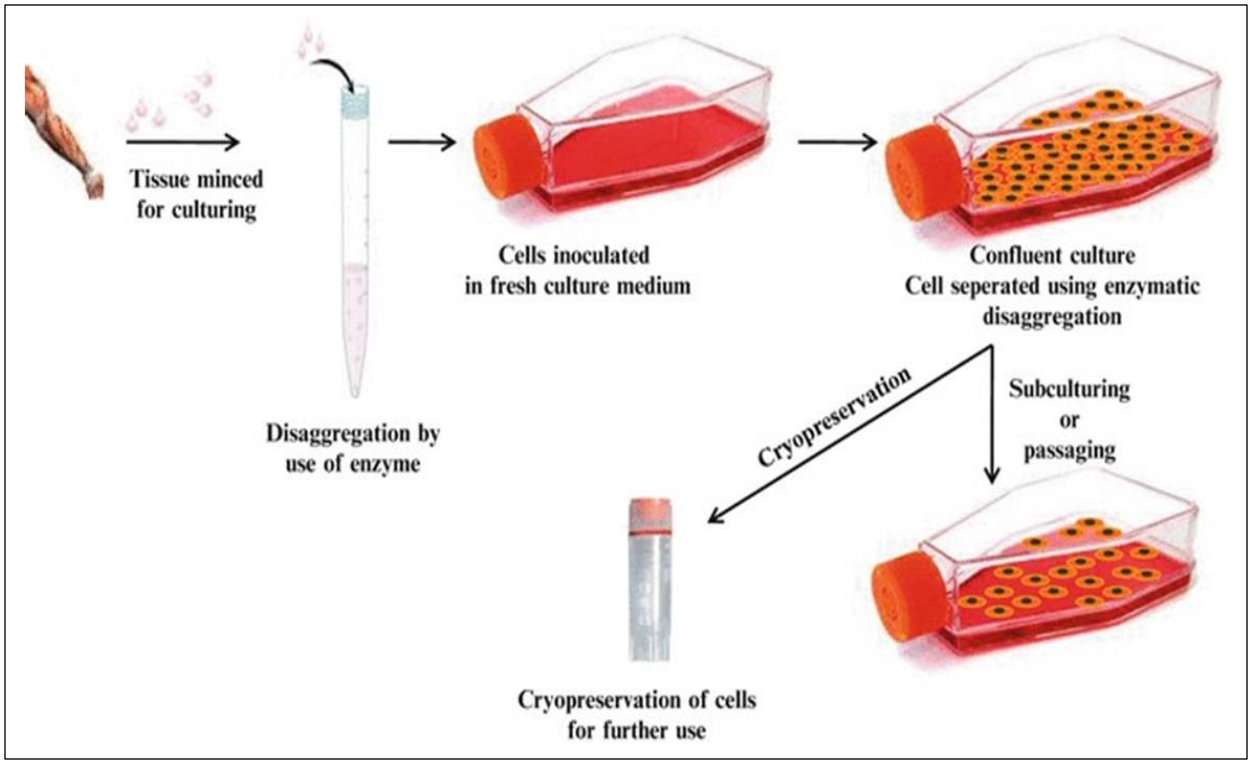
Types of cell culture

1- Primary culture

- Primary culture is the cell culture system that is formed by culture cells directly obtained from tissue and proliferate them until they occupy all of the available substrate (i.e. reach Confluence).
- For primary culture, the first step is to isolate the tissue from organ and splitting of cells from tissues.
- Then grow cells in freshly prepared cell specific medium and incubate the flask containing cells in incubator providing suitable environmental condition for the growth of cells.
- After the first subculture, the primary culture is known as a cell line or subclone.

2- Secondary culture

- When cells from primary culture on confluency are isolated and cultured in new media, it is called as secondary culture.
- Secondary culture is also known as subculture or splitting of cells.
- Subculture allows fresh nutrients and more space for the expansion of the cells.
- Cells from primary culture are splits by trypsin/EDTA treatment.
- Trypsin which is a serine protease digest the extracellular protein or matrix protein so that cells get free.
- EDTA which is a chelating agent chelates calcium ion because calcium helps in cell adhesion.



Requirement of cell culture

- 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.

Cell culture applications:

1. Excellent model systems for studying:

✓ The normal physiology and biochemistry of cells.

✓ The effects of drugs and toxic compounds on the cells.

✓ Mutagenesis and carcinogenesis.

2. Used in drug screening and development.

3. Large scale manufacturing of biological compounds.

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 reason 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 splitted)