Cell Culture: Uses, Techniques and Best Practice

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What is Cell Culture?

Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established.

Major developments in cell culture technology

- First development was the use of antibiotics which inhibits the growth of contaminants.
- Second was the use of trypsin to remove adherent cells to subculture further from the culture vessel
- Third was the use of chemically defined culture medium.

What is the cell culture used for?

- Model systems for

  Studying basic cell biology, interactions between disease causing agents and cells, effects of drugs on cells, process and triggering of aging & nutritional studies

- Toxicity testing

  Study the effects of new drugs

- Cancer research

  Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells

Contd....

- Virology

  Cultivation of virus for vaccine production, also used to study there infectious cycle.

- Genetic Engineering

  Production of commercial proteins, large scale production of viruses for use in vaccine production e.g. polio, rabies, chicken pox, hepatitis B & measles

- Gene therapy

  Cells having a functional gene can be replaced to cells which are having non-functional gene

Tissue culture

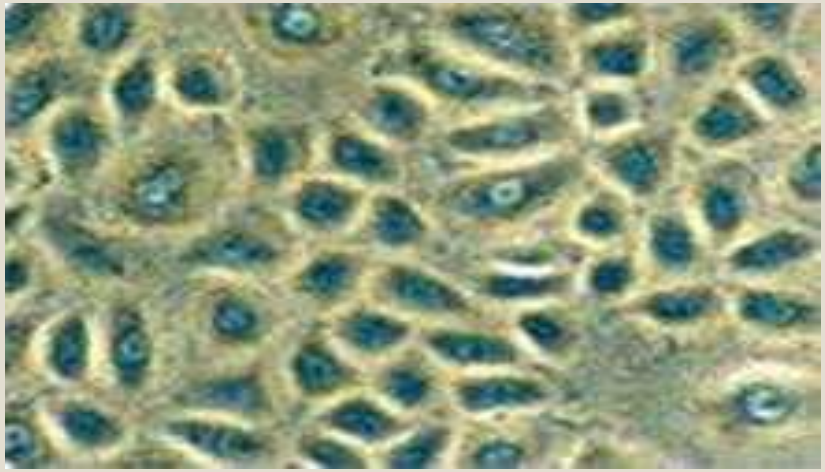
- In vitro cultivation of organs, tissues & cells at defined temperature using an incubator & supplemented with a medium containing cell nutrients & growth factors is collectively known as tissue culture

- Different types of cell grown in culture includes connective tissue elements such as fibroblasts, skeletal tissue, cardiac, epithelial tissue (liver, breast, skin, kidney) and many different types of tumor cells.

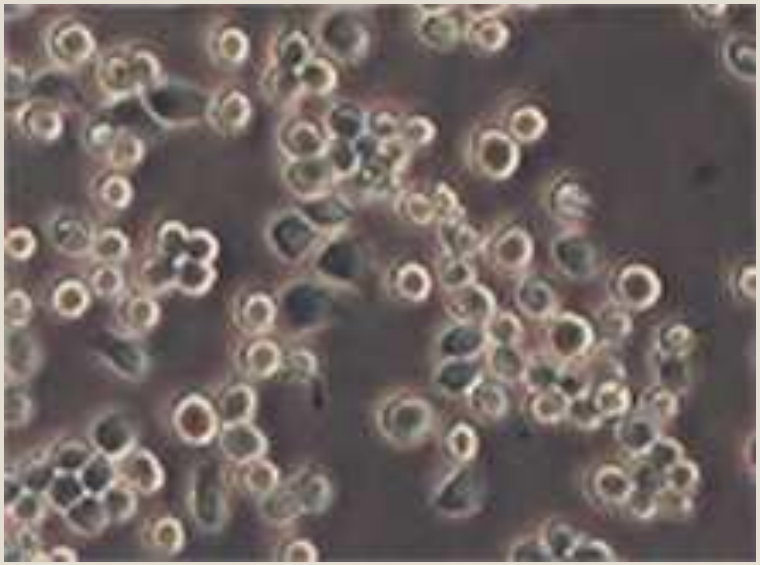
Types of cells

On the basis of morphology (shape & appearance) or on their functional characteristics. They are divided into three.

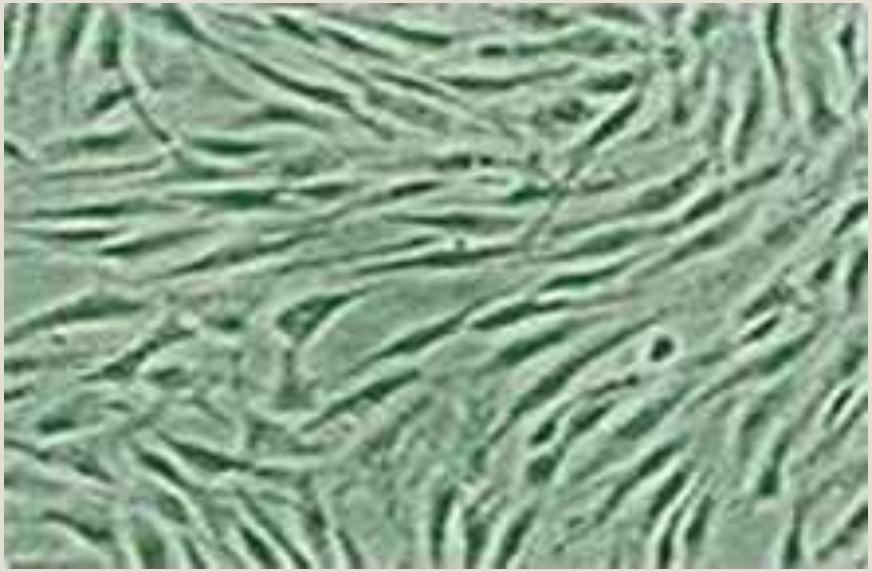
- Epithelial like-attached to a substrate and appears flattened and polygonal in shape



- Lymphoblast like- cells do not attach remain in suspension with a spherical shape



- Fibroblast like- cells attached to an substrate appears elongated and bipolar



Culture media

- Choice of media depends on the type of cell being cultured
- Commonly used Medium are GMEM, EMEM,DMEM etc.
- Media is supplemented with antibiotics viz. penicillin, streptomycin etc.
- Prepared media is filtered and incubated at 4 C

Why sub culturing?

- Once the available substrate surface is covered by cells (a confluent culture) growth slows & ceases.
- Cells to be kept in healthy & in growing state have to be sub-cultured or passaged
- It's the passage of cells when they reach to 80-90% confluency in flask/dishes/plates
- Enzyme such as trypsin, dipase, collagenase in combination with EDTA breaks the cellular glue that attached the cells to the surface

Adherent cells

- Cells which are anchorage dependent
- Cells are washed with PBS (free of ca & mg) solution.
- Add enough trypsin/EDTA to cover the monolayer
- Incubate the plate at 37 C for 1-2 mts
- Tap the vessel from the sides to dislodge the cells
- Add complete medium to dissociate and dislodge the cells
- with the help of pipette which are remained to be adherent
- Add complete medium depends on the subculture
- requirement either to 75 cm or 175 cm flask

Suspension cells

- Easier to passage as no need to detach them
- As the suspension cells reach to confluency
- Asceptically remove 1/3rd of medium
- Replaced with the same amount of pre-warmed medium

Cell toxicity

- Cytotoxicity causes inhibition of cell growth
- Observed effect on the morphological alteration in the cell layer or cell shape
- Characteristics of abnormal morphology is the giant cells, multinucleated cells, a granular bumpy appearance, vacuoles in the cytoplasm or nucleus

Working with cryopreserved cells

- Vial from liquid nitrogen is placed into 37 C water bath, agitate vial continuously until medium is thawed
- Centrifuge the vial for 10 mts at 1000 rpm at RT, wipe top of vial with 70% ethanol and discard the supernatant
- Resuspend the cell pellet in 1 ml of complete medium with 20% FBS and transfer to properly labeled culture plate containing the appropriate amount of medium
- Check the cultures after 24 hrs to ensure that they are attached to the plate
- Change medium as the colour changes, use 20% FBS until the cells are established

Freezing cells for storage

- Remove the growth medium, wash the cells by PBS and remove the PBS by aspiration
- Dislodge the cells by trypsin-versene
- Dilute the cells with growth medium
- Transfer the cell suspension to a 15 ml conical tube, centrifuge at 200g for 5 mts at RT and remove the growth medium by aspiration
- Resuspend the cells in 1-2ml of freezing medium
- Transfer the cells to cryovials, incubate the cryovials at -80 C overnight
- Next day transfer the cryovials to Liquid nitrogen

Cell viability

- Cell viability is determined by staining the cells with trypan blue
- As trypan blue dye is permeable to non-viable cells or death cells whereas it is impermeable to this dye
- Stain the cells with trypan dye and load to haemocytometer and calculate % of viable cells

  - % of viable cells= $\frac{\text{Nu. of unstained cells}}{\text{total nu. of cells}} \times 100$

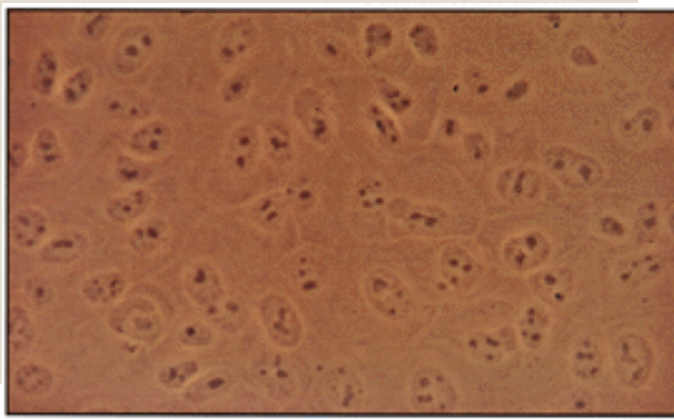
Common cell lines

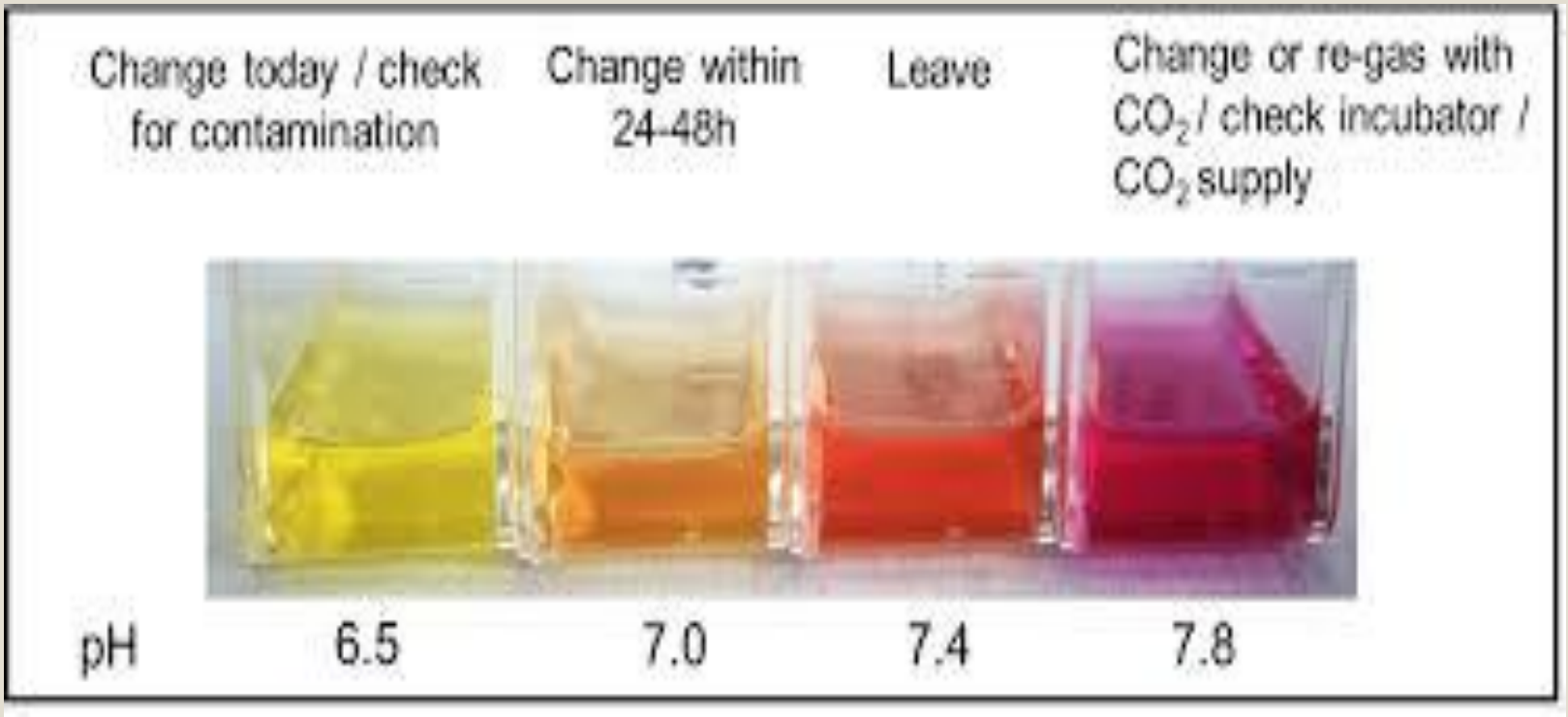
- Human cell lines
- -MCF-7 breast cancer
- HL 60 Leukemia
- HEK-293 Human embryonic kidney
- HeLa Henrietta lacks
- Primate cell lines
- Vero African green monkey kidney epithelial cells
- Cos-7 African green monkey kidney cells
- And others such as CHO from hamster, sf9 & sf21 from insect cells

Contaminants of cell culture

Cell culture contaminants of two types

- Chemical-difficult to detect caused by endotoxins, plasticizers, metal ions or traces of disinfectants that are invisible
- Biological-cause visible effects on the culture they are mycoplasma, yeast, bacteria or fungus or also from cross-contamination of cells from other cell lines




Change today / check for contamination
Change within 24-48h
Leave
Change or re-gas with CO_2 / check incubator / CO_2 supply
pH
6.5
7.0
7.4
7.8

Effects of Biological Contamination's

- They competes for nutrients with host cells
- Secreted acidic or alkaline by-products ceses the growth of the host cells
- Degraded arginine & purine inhibits the synthesis of histone and nucleic acid
- They also produces H_2O_2 which is directly toxic to cells

Detection of contaminants

- In general indicators of contamination are turbid culture media, change in growth rates, abnormally high pH, poor attachment, multi-nucleated cells, graining cellular appearance, vacuolization, inclusion bodies and cell lysis
- Yeast, bacteria & fungi usually shows visible effect on the culture (changes in medium turbidity or pH)
- Mycoplasma detected by direct DNA staining with intercalating fluorescent substances e.g. Hoechst 33258
- Mycoplasma also detected by enzyme immunoassay by specific antisera or monoclonal abs or by PCR amplification of mycoplasmal RNA
- The best and the oldest way to eliminate contamination is to discard the infected cell lines directly

Basic equipment used in cell culture

- Laminar cabinet-Vertical are preferable
- Incubation facilities- Temperature of 25-30 C for insect & 37 C for mammalian cells, co2 2-5% & 95% air at 99% relative humidity. To prevent cell death incubators set to cut out at approx. 38.5 C
- Refrigerators- Liquid media kept at 4 C, enzymes (e.g. trypsin) & media components (e.g. glutamine & serum) at -20 C
- Microscope- An inverted microscope with 10x to 100x magnification
- Tissue culture ware- Culture plastic ware treated by polystyrene

Basic aseptic conditions

- If working on the bench use a Bunsen flame to heat the air surrounding the Bunsen
- Swab all bottle tops & necks with 70% ethanol
- Flame all bottle necks & pipette by passing very quickly through the hottest part of the flame
- Avoiding placing caps & pipettes down on the bench; practice holding bottle tops with the little finger
- Work either left to right or vice versa, so that all material goes to one side, once finished
- Clean up spills immediately & always leave the work place neat & tidy

Safety aspect in cell culture

- Possibly keep cultures free of antibiotics in order to be able to recognize the contamination
- Never use the same media bottle for different cell lines. If caps are dropped or bottles touched unconditionally touched, replace them with new ones
- Necks of glass bottles prefer heat at least for 60 secs at a temperature of 200 C
- Switch on the laminar flow cabinet 20 mts prior to start working
- Cell cultures which are frequently used should be subcultered & stored as duplicate strains

Other key facts…….?

- Use actively growing cells that are in their log phase of growth, which are 80-90% viable
- Keep exposure to trypsin at a minimum
- Handle the cells gently. Do not centrifuge cells at high speed or roughly re-suspend the cells

Thank you

Adapt it with your needs and it will capture all the audience attention.