Cell and Tissue Culture Principles

Neda Esfandiari

Department of Nanobiotechnology

Faculty of Life Sciences and Biotechnology

Shahid Beheshti University

Ne_esfandiari@sbu.ac.ir

http://facultymembers.sbu.ac.ir/esfandiari

What is a cell culture?

- *Cell differentiation* ⟺ *cell function*
- Tissue culture is the general name for the removal of cells, tissues or organs from an animal placement into **artificial environment** conductive to growth.
- In vivo ⟹ in vitro
- the term ***tissue culture*** is used as a generic term to include organ culture and cell culture.

Organ culture

- *organ culture* will always imply a three-dimensional culture of undisaggregated tissue retaining some or all of the histological features of the tissue in vivo.

Cell culture

- culture derived from dispersed cells taken from original tissue, from a primary culture, or from a cell line or cell strain by enzymatic, mechanical, or chemical disaggregation.

Histotypic culture

- cells have been reaggregated or grown to recreate a three-dimensional structure

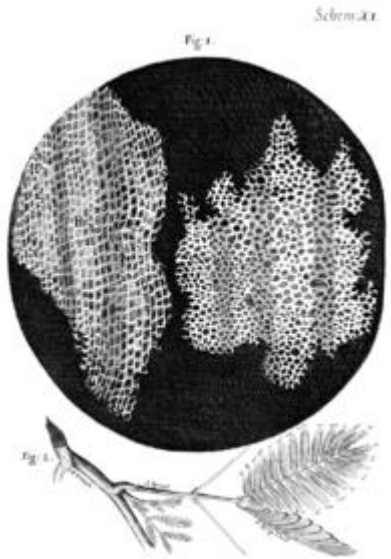
Organotypic culture

- In this case, the cells from different lineages are put together to create a component of an organ in the laboratory.

History of cell culture

Robert Hooke

1665

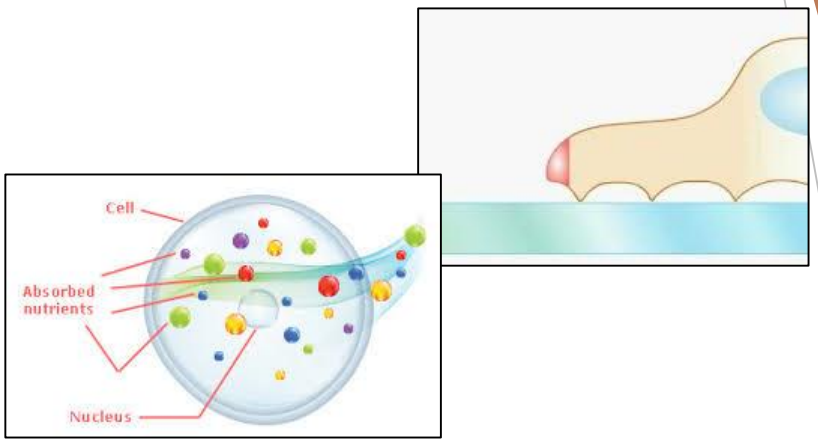


Antonie Philips van Leeuwenhoek

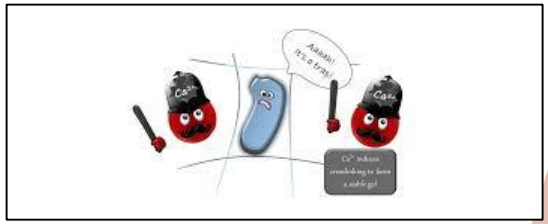
Cell theory
1855

Harrison [1907] chose the frog as a source of tissue : cold-blooded animal

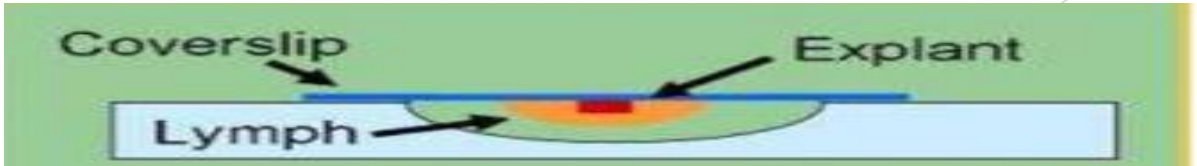
- *Adhesion* of *cells*

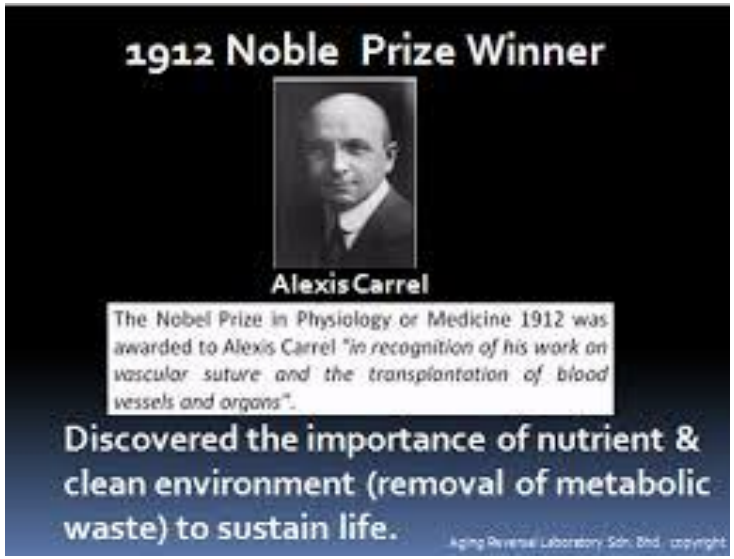


- *Food*



- The growth rate is relatively low



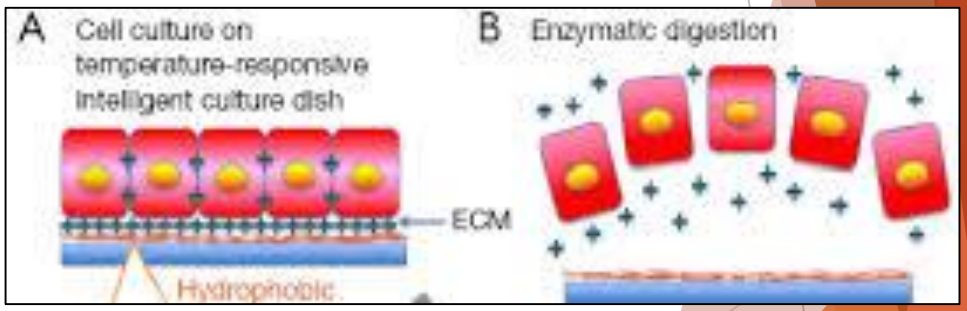


Carrel, Aseptic technique

Trypsin
(1916-1950)

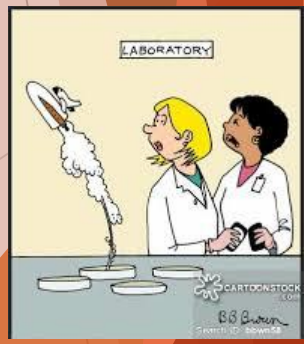
- Trypsinization and subculture

- remove adherent cells to subculture further from the culture vessel



Cell culture application

- As a model systems
- Cancer studies
- Virology
- Cytotoxicity
- vaccine production
- Production of recombinant proteins
- Tissue replacement
- Genetic counseling
- Gene therapy
- Medicine



Advantages of Tissue Culture

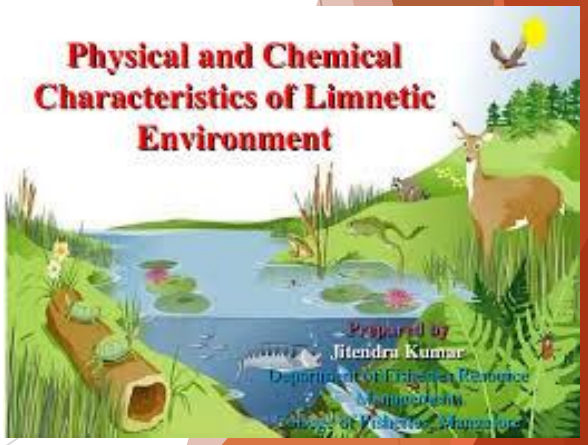
1-Control of **environment**

- physiochemical environment

(pH, temperature, osmotic pressure, and O2 and CO2 tension)

- physiological environment

(Hormones and nutrition condition)



Advantages of Tissue Culture

2-Homogenecity

3 -Repeatability

3- Economy

4- Preservation

5- Reduction of animal use

LIMITATIONS

- **Expertise** : Sterile handling(microbial contamination)

provision of a complex environment simulating blood plasma

- **Cost** : Capital equipment for scale-up

Medium, serum, cells (1-10 gr cells)

- **Origin of Cells** : **Identification of cell type**

Avoidance of cross- contamination

TYPES OF TISSUE CULTURE

Organ culture

Primary culture

Cell culture

Organ culture

- Parts of an organ or a whole organ can be cultured in vitro

- Advantages

  Cells interaction

- Disadvantages

  Oxygen

  Do not grow rapidly

Primary culture

- Cells when surgically or enzymatically removed from an organism and placed in suitable culture environment will attach and grow are called as primary culture.

- Disadvantages

  Primary cells have a **finite life** span.

  adaptation

  contamination

- Advantages

  Normal Characteristics of organ are maintained

  Oxygen

ISOLATION OF THE TISSUE primary culture

- 1- Mouse Embryo
- 2- chick Embryo
- 3- Human Biopsy

Mouse Embryo:

Mate → (zero days)

13 days (

m uterus

g the embryos

culture

Chick Embryo

Incubate the eggs at 38.5 ° C ➡ 8 days

Crack the top of the shell

air sac

Lift out the embryo

Transfer the embryo to a petri dish contai

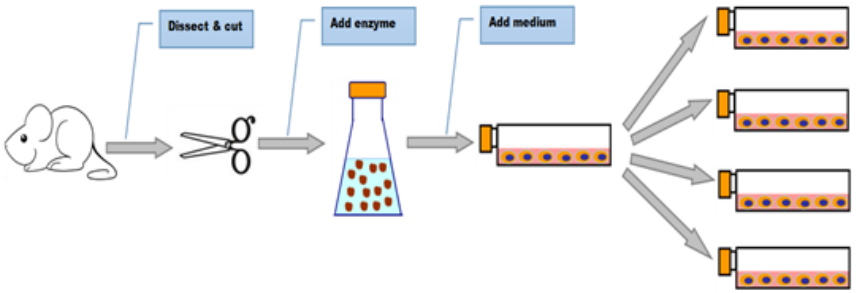
Chopping (enzyme-mechanical)

culture

Human Biopsy Material

biopsy sampling is usually performed for diagnostic purpose

- Proteolytic activity of enzymes (*Trypsin*, *Collagenases*)
- Centrifuge
- Aseptic technique (EtOH 70%)



Cell culture

- Lost their apparent

Cell passage

Genetic diversity

Cell line

cell freezing

- After the first subculture, the primary culture becomes known as a **cell line** **(secondary culture)**

Cell culture

1- ***finite cells*** **:**

- **thirty cycles** of division **(stock)**

- growth rate (24-96 h)
- Purchase and maintenance
- Information about nutritional and biological activities

Contact inhibition

2- Immortalised cells:

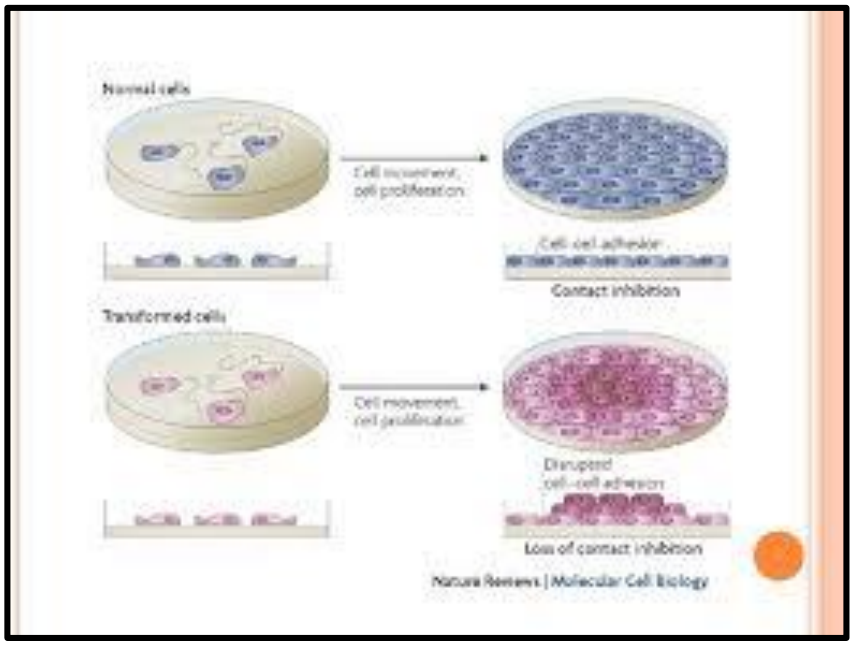
a population of cells from a multicellular organism which would normally not proliferate indefinitely but, due to **mutation** have evaded normal cellular senescence and instead can keep undergoing division.

- ✓ The main advantage of using an immortal cell line for **research** is its immortality.

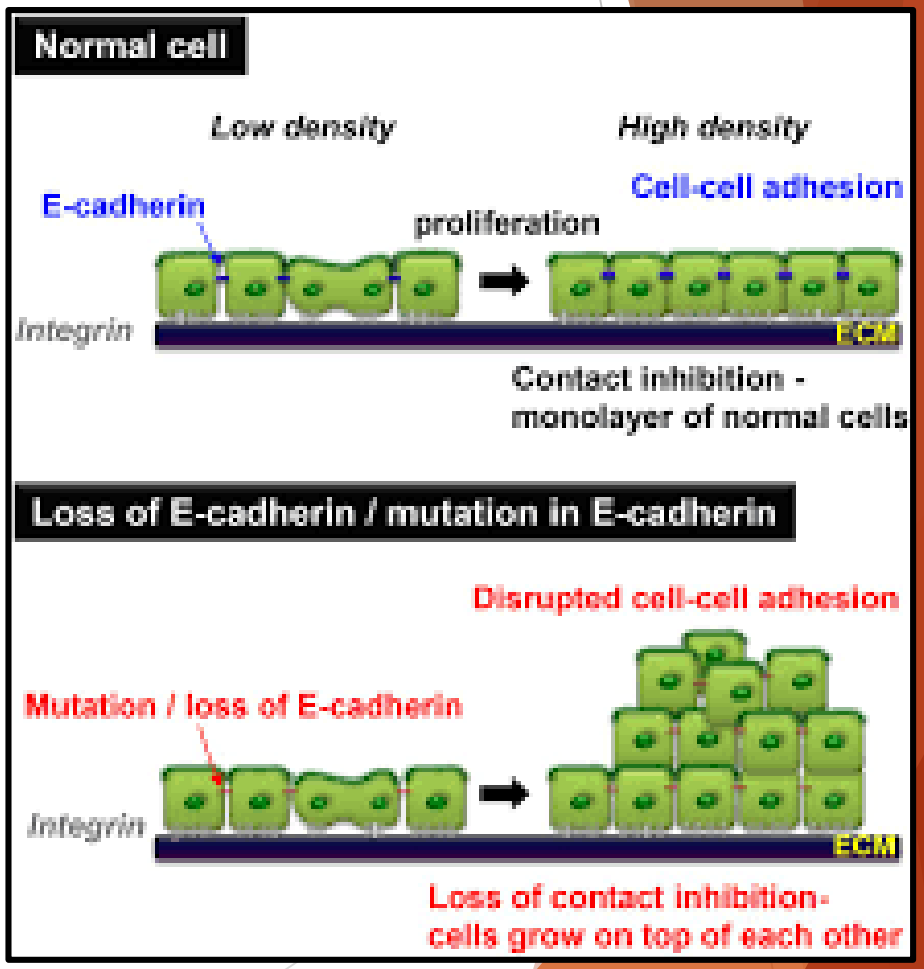
Cancer or stem cells

- ✓ High growth rate (12-24 h)

cell lines can change genetically over multiple passages.



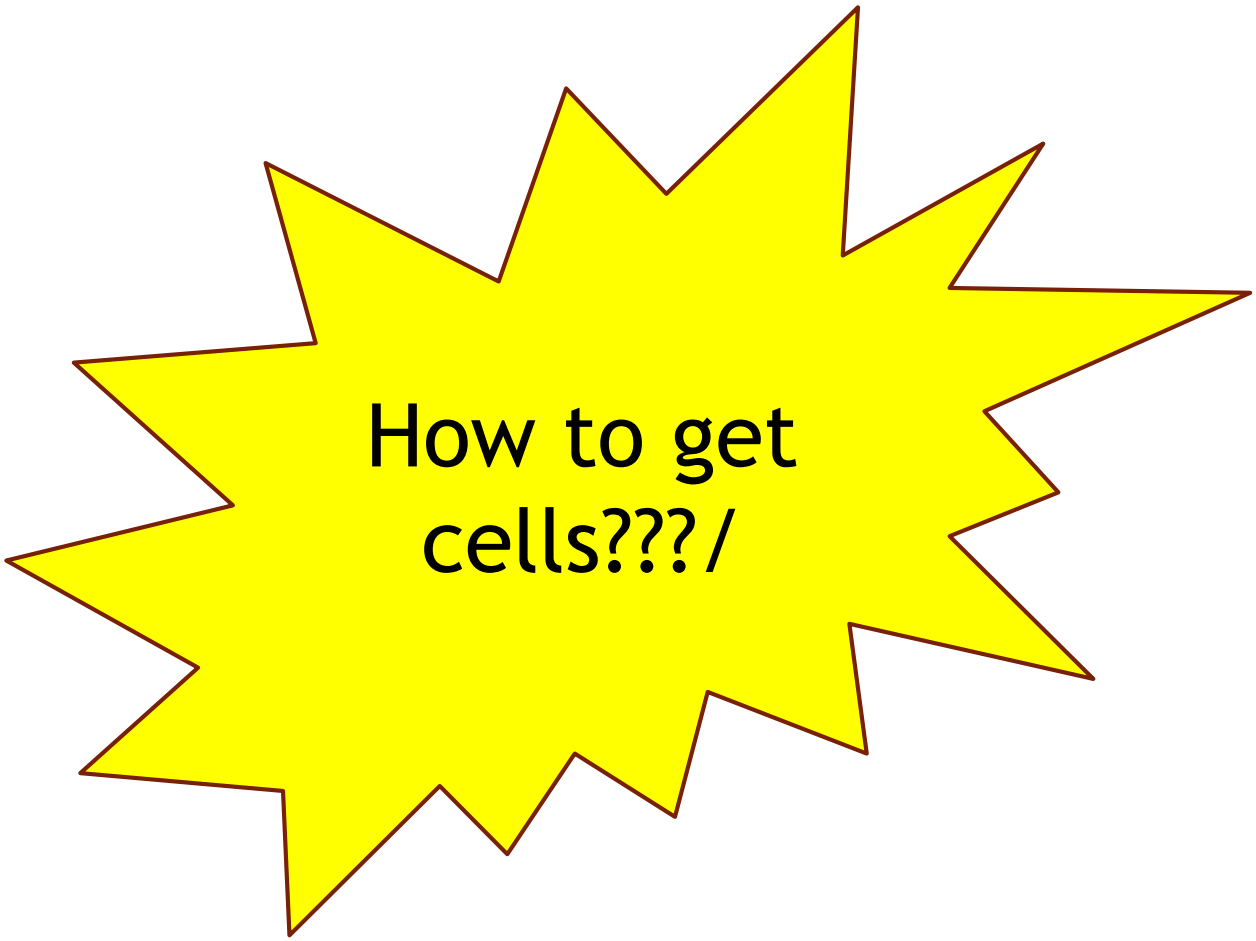
Contact inhibition



Production of Immortalised cells

- Isolation from cancer cells
- Virus infection
- Artificial expression of key proteins (**telomerase**)
- Hybridoma technology

Biology of cultured cells


How to get cells???/

Types of cells :

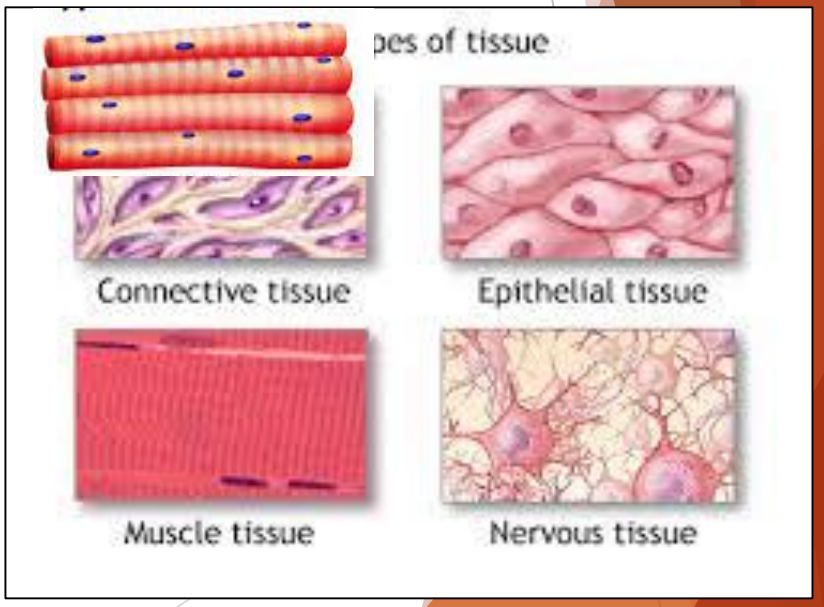
1- Epithelium cells

2- Connective cells (*fibroblast* cells)

3- Muscle cells (myoblast)

4- Nervous cells

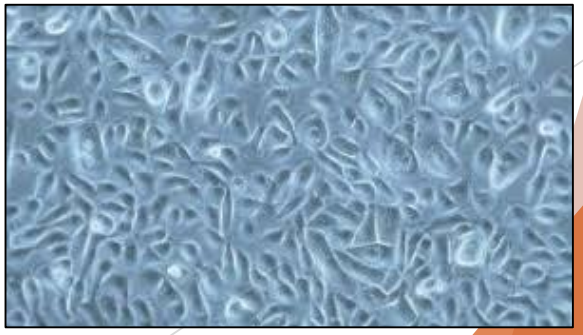
5- blood cells



How to select the cells?

Environment conditions

- Substrate (solid, semi- solid, liquid)
- Contact with other cells
- Physiological and Physiochemical of the environment

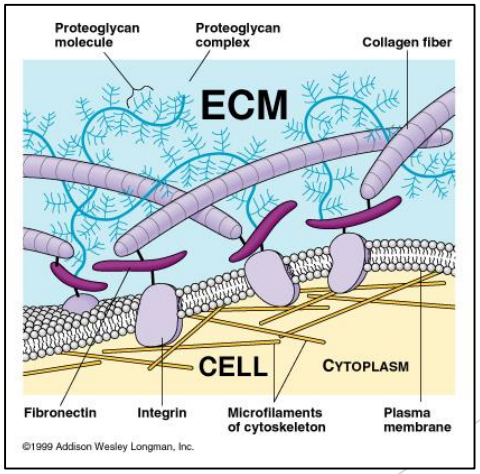


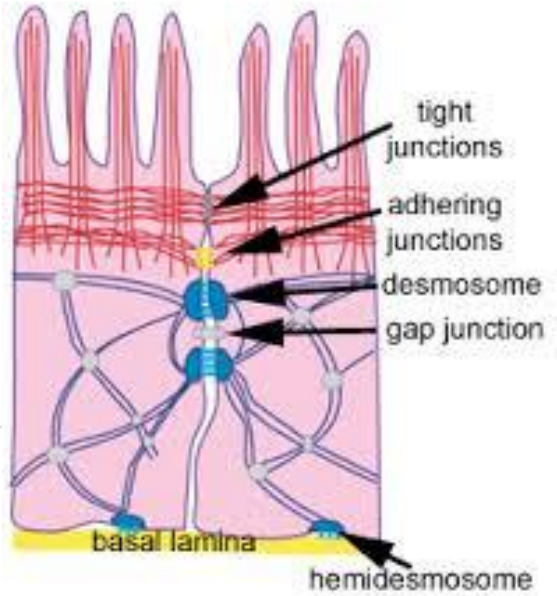
CELL ADHESION

- They will need to attach and spread out on the substrate before they will start to proliferate.

- Electrostatic charge density (glass or polystyrene)

- **Protease (Epithelial cells, Endothelium cells, Mesenchymal cells)**



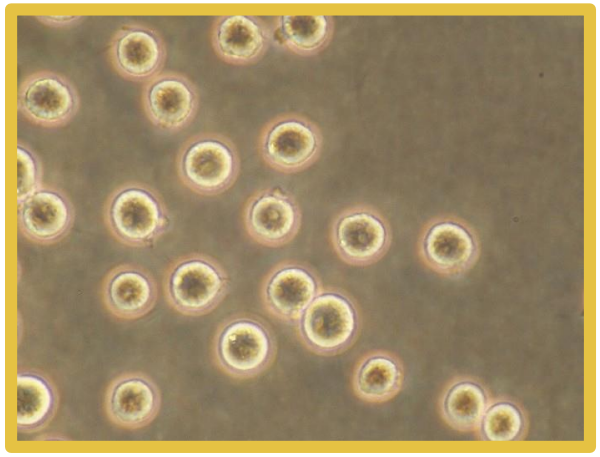


Cells divided into two main types (attach to a surface):

1- Suspension cell culture

(Anchorage-independent):

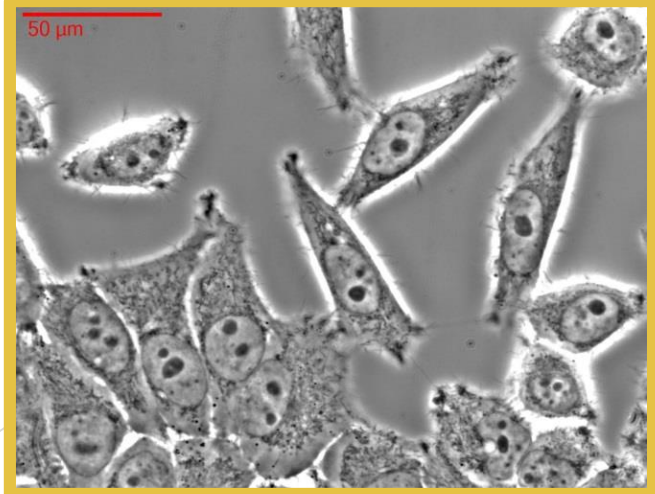
- Grow rapidly
- No passage
- No trypsin
- Easier maintenance



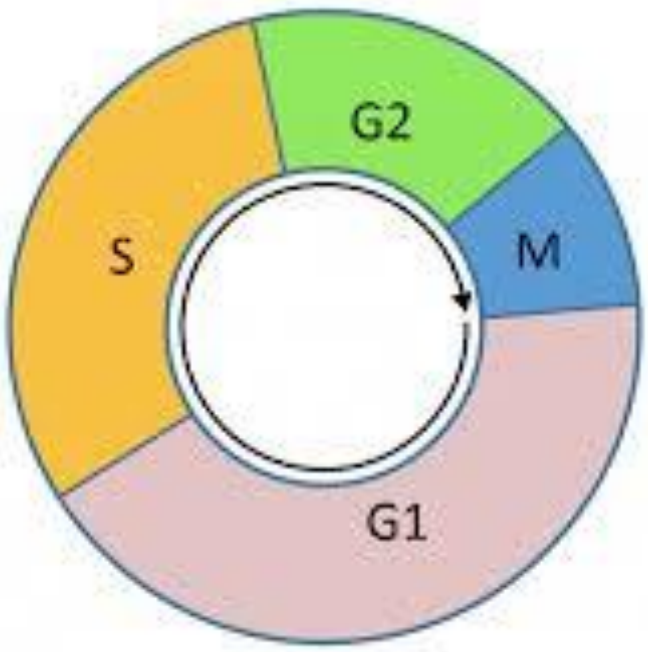
2- Adherent cell culture (Anchorage-dependent) monolayer

- Most cells from **solid tissues** grow as ⟹ adherent **monolayer**
- Growth is limited by surface area (***contact inhibition***) Cellular mobility

Cell shapes ⟹ type of tissue



Cell cycle



G1 - Growth

S - DNA synthesis

G2 - Growth and preparation for mitosis

M - Mitosis (cell division)

Growth curve and Growth Cycle

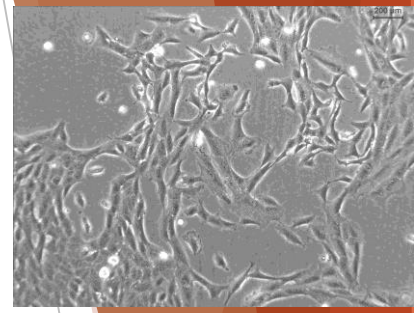
1- Lag Phase

- This is the time following subculture and reseeding during which there is little evidence of an increase in **cell number**
- It is a period of **adaptation** during which the cell replaces and **attaches to the surface**, and **spreads out**
- The length of the log phase depends on the **growth rate** and the **number** of the cells during subculture

2- Log Phase

- This is the period of exponential **increase in cell number**

- It is the **optimal time for sampling** since the population is at its most uniform and viability is high

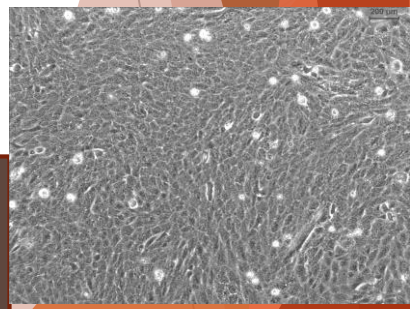


70-80% confluence

3- Plateau Phase

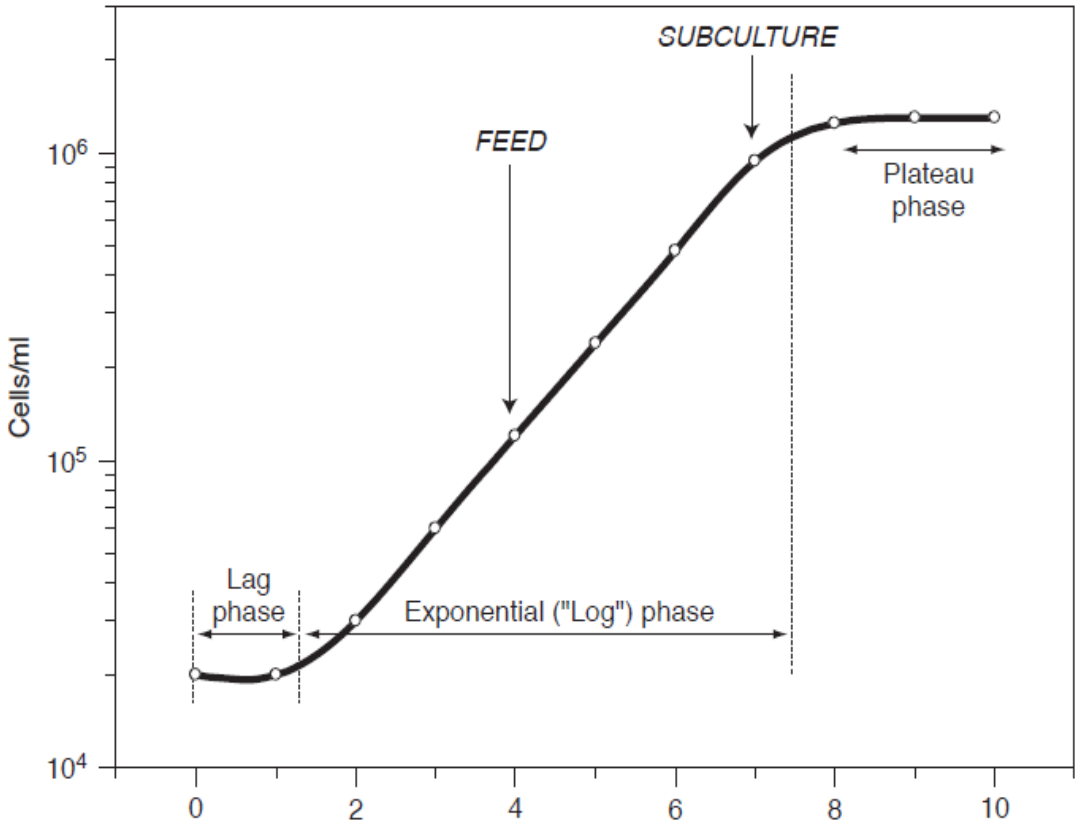
- Toward the end of the log phase, the culture **becomes confluent**

100% confluence



4- Death phase

confluence : an estimate of the number of adherent cell in a culture dishes or a flask, referring to the proportion of the surface which is covered by cells.


SUBCULTURE
FEED
Plateau phase
Lag phase
Exponential ("Log") phase
Cells/ml
10^6
10^5
10^4
0
2
4
6
8
10