

Cell Culture Systems

Course 1

Cell Types, Organelles and Cell Culture

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What is a Cell?

- All living things are made from one or more cells.
- A **cell** is the **simplest unit of life and they are responsible for keeping an organism alive and functioning.**

CELL ORGANIZATION

Cells



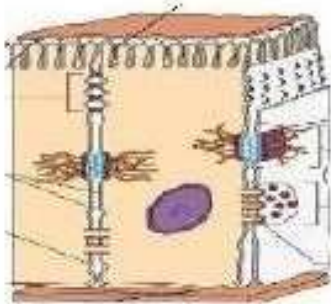
Tissues



Organs



System



Epithelial cell



Epithelial tissue



Stomach



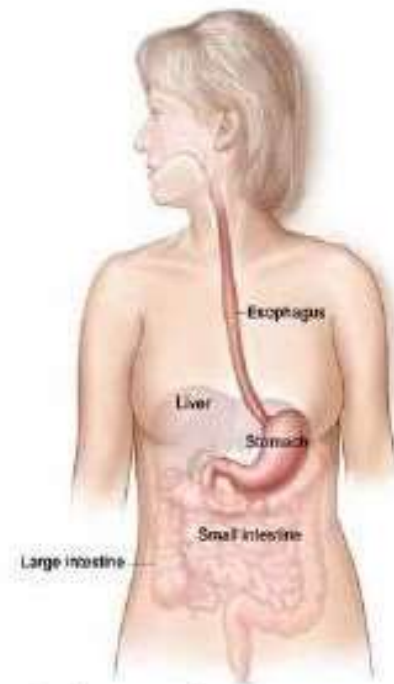
**Smooth
muscle cell**



Epithelial tissue



**Small
intestine**



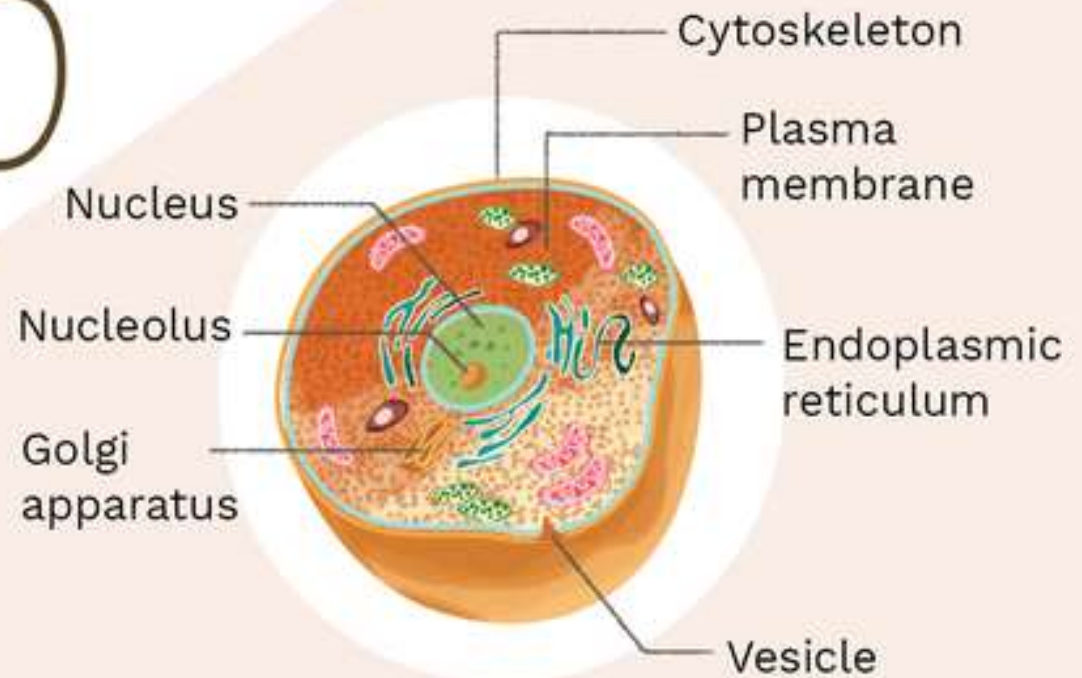
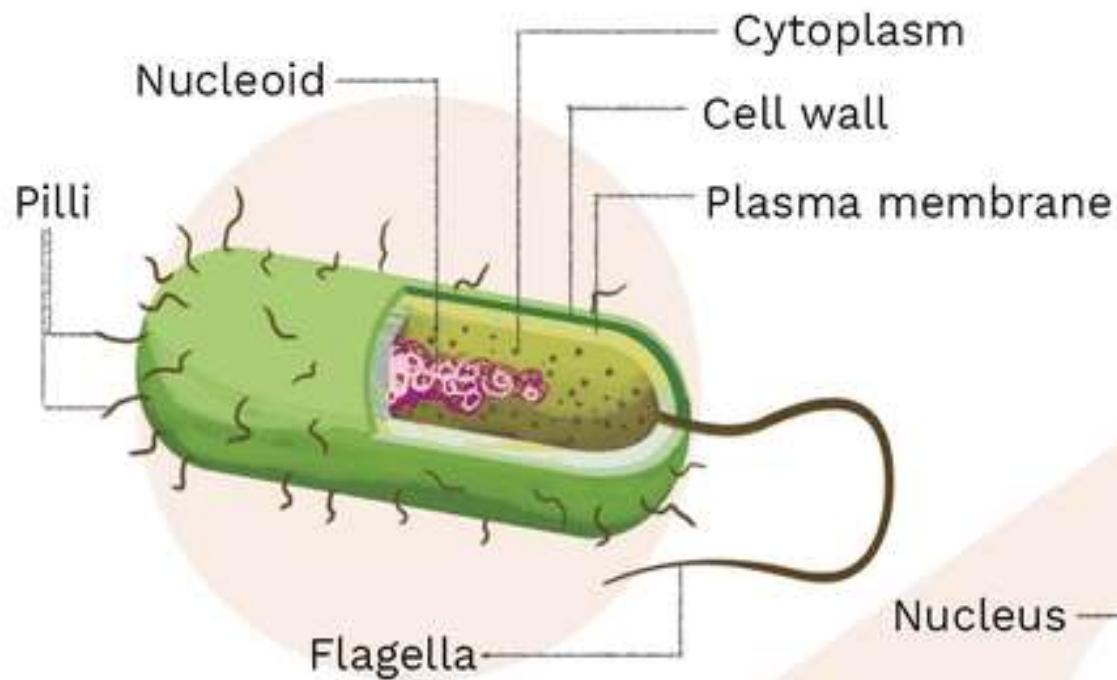
Digestive system

- There is a massive variety of different types of cells but they all have some common characteristics.
- Almost every different type of cell contains
- genetic material, a membrane and cytoplasm. Cells also have many features such as other and as s ribosomes that perform specific functions.

- All cells can be divided into one of two classifications: prokaryotic cells and eukaryotic cells.
- Prokaryotic cells are found in bacteria and archaea.
- Eukaryotic cells are found in organisms from the domain Eukaryota which includes animals, plants, fungi and protists.

- The main difference between prokaryotic cells and eukaryotic cells is the presence of a nucleus and organelles.
- Prokaryotic cells do not have either a nucleus or organelles. The word prokaryotic can be translated to mean 'before nucleus'.

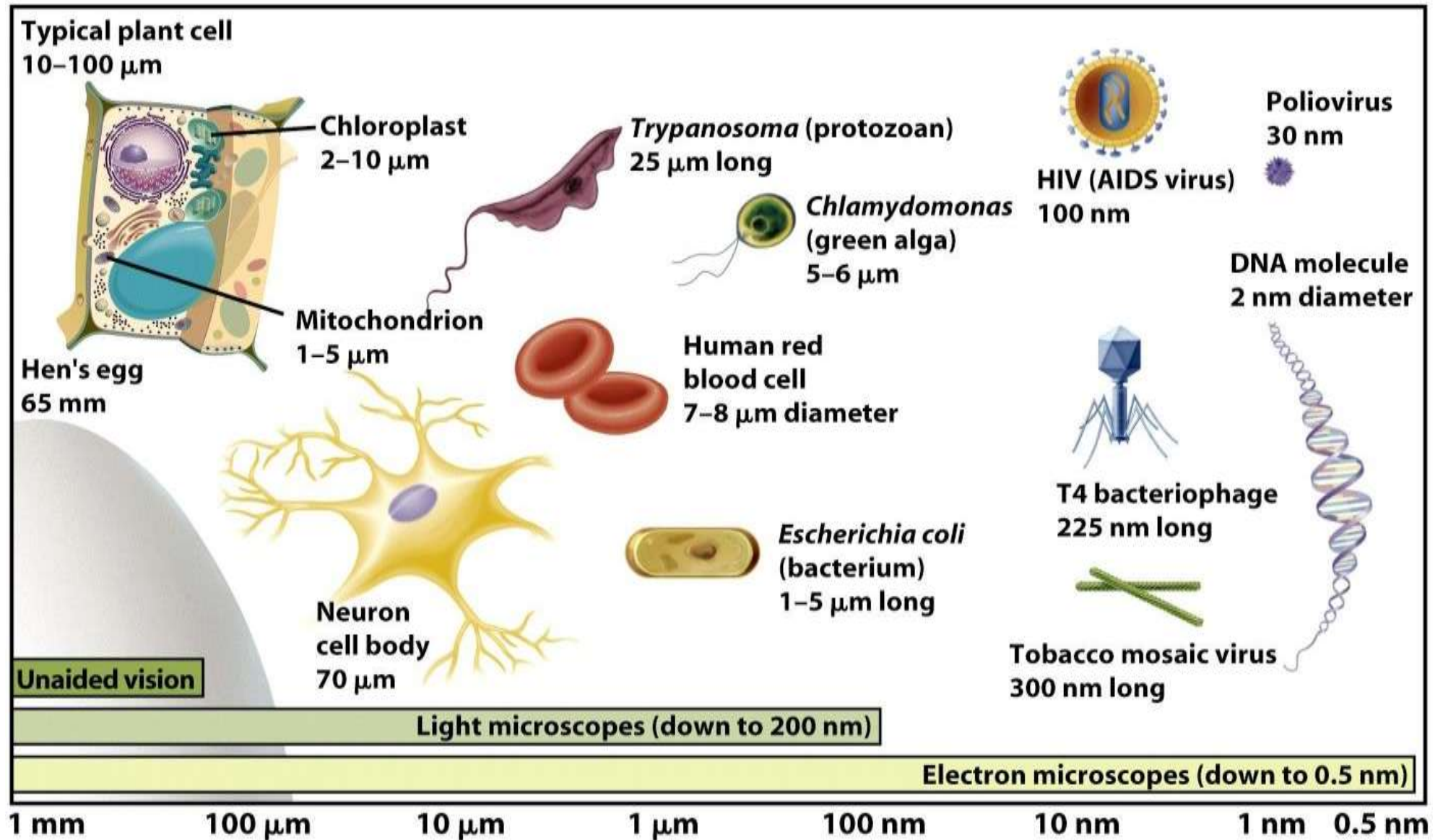
PROKARYOTIC CELL



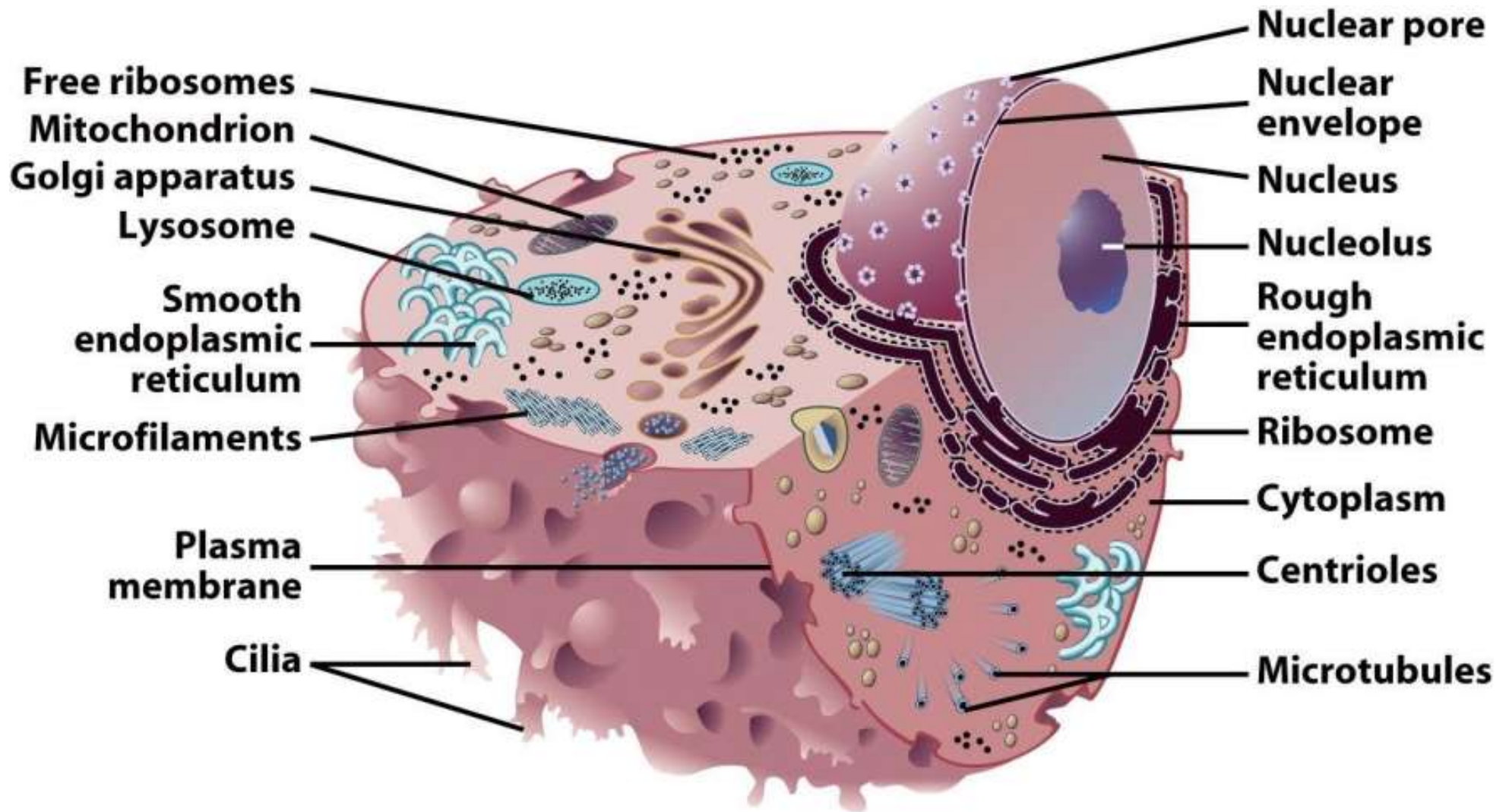
EUKARYOTIC CELL

Characteristic	Prokaryotic cell	Eukaryotic cell
Size of cell	Typically 0.2-2.0 μ m in diameter	Typically 10-100 μ m in diameter
Example	Bacteria and Archaea	Animals and Plants
Nucleus	Absent	Present
Membrane-enclosed organelles	Absent	Present; examples include lysosomes, Golgi complex, endoplasmic reticulum, mitochondria & chloroplasts
Flagella	Consist of two protein building blocks	Complex; consist of multiple microtubules
Cell wall	Usually present; chemically complex	Only in plant cells and fungi (chemically simpler)
Plasma membrane with steroid	Usually no	Yes
Cytoplasm	No cytoskeleton or cytoplasmic streaming	Cytoskeleton; cytoplasmic streaming
Ribosomes	Smaller	Larger
Cell division	Binary fission	Mitosis
Number of chromosomes	One, but not true chromosome	More than one
Sexual reproduction	No meiosis; transfer of DNA fragments only (conjugation)	Involves meiosis

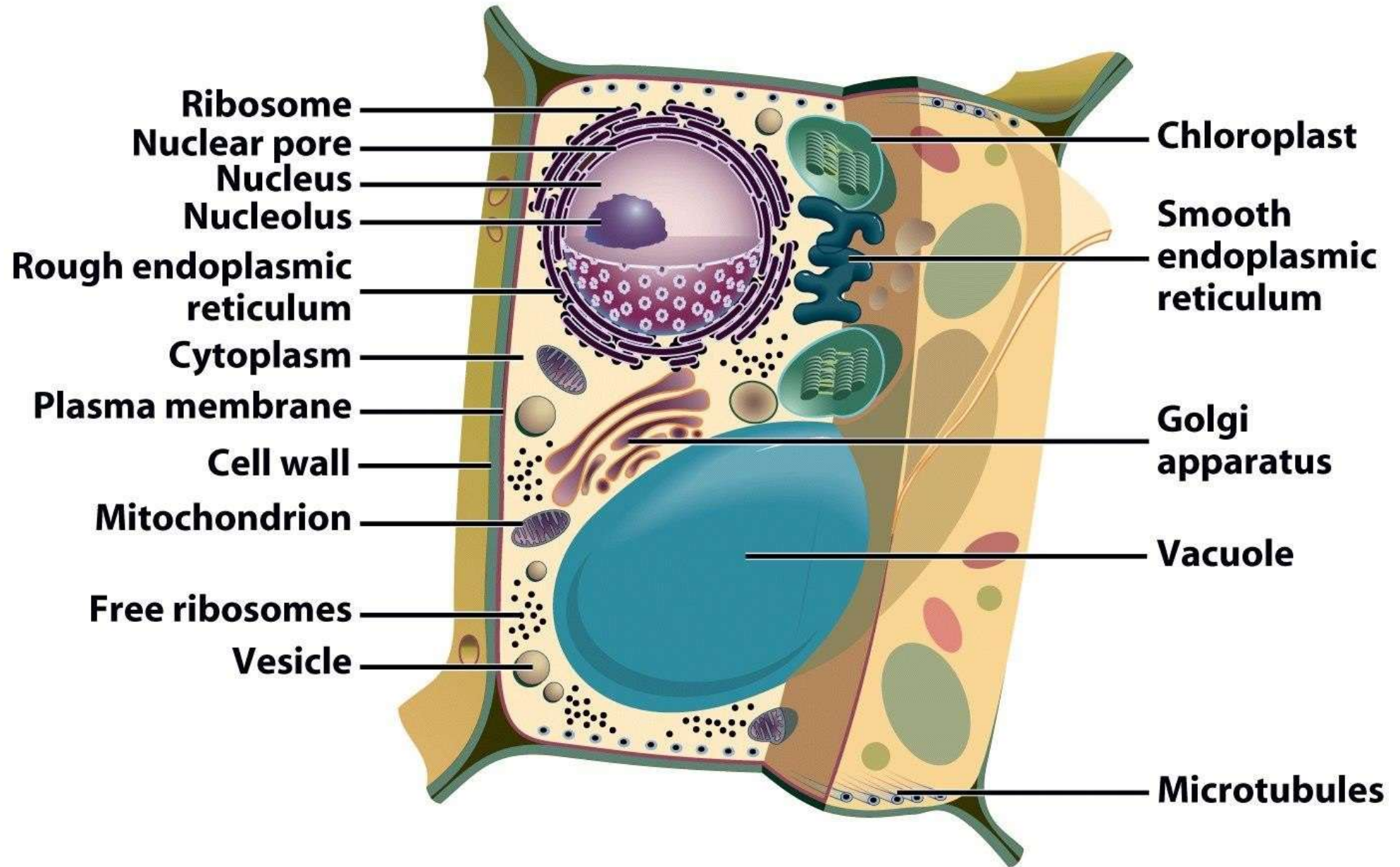
Cell Size



Representative Animal Cell

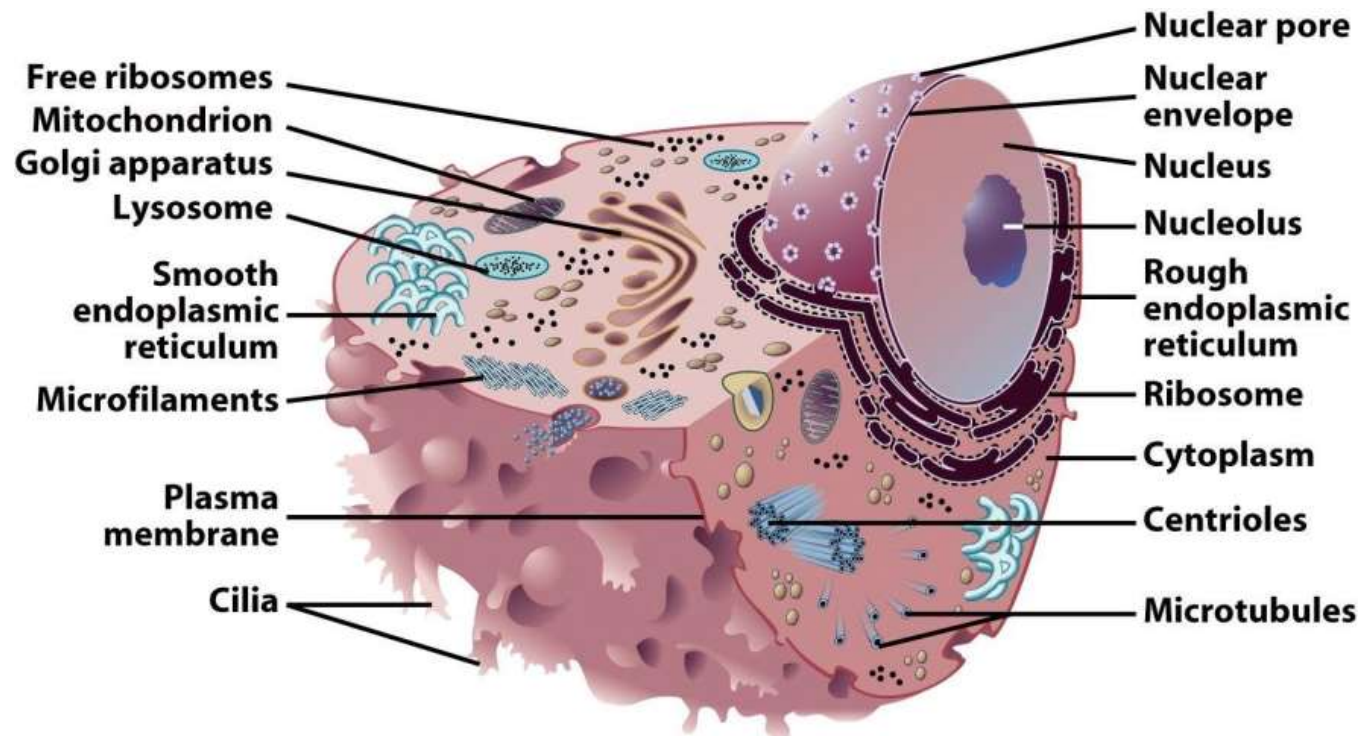


Representative Plant Cell



Organelles

- Cellular machinery
- Two general kinds
 - Derived from membranes
 - Bacteria-like organelles

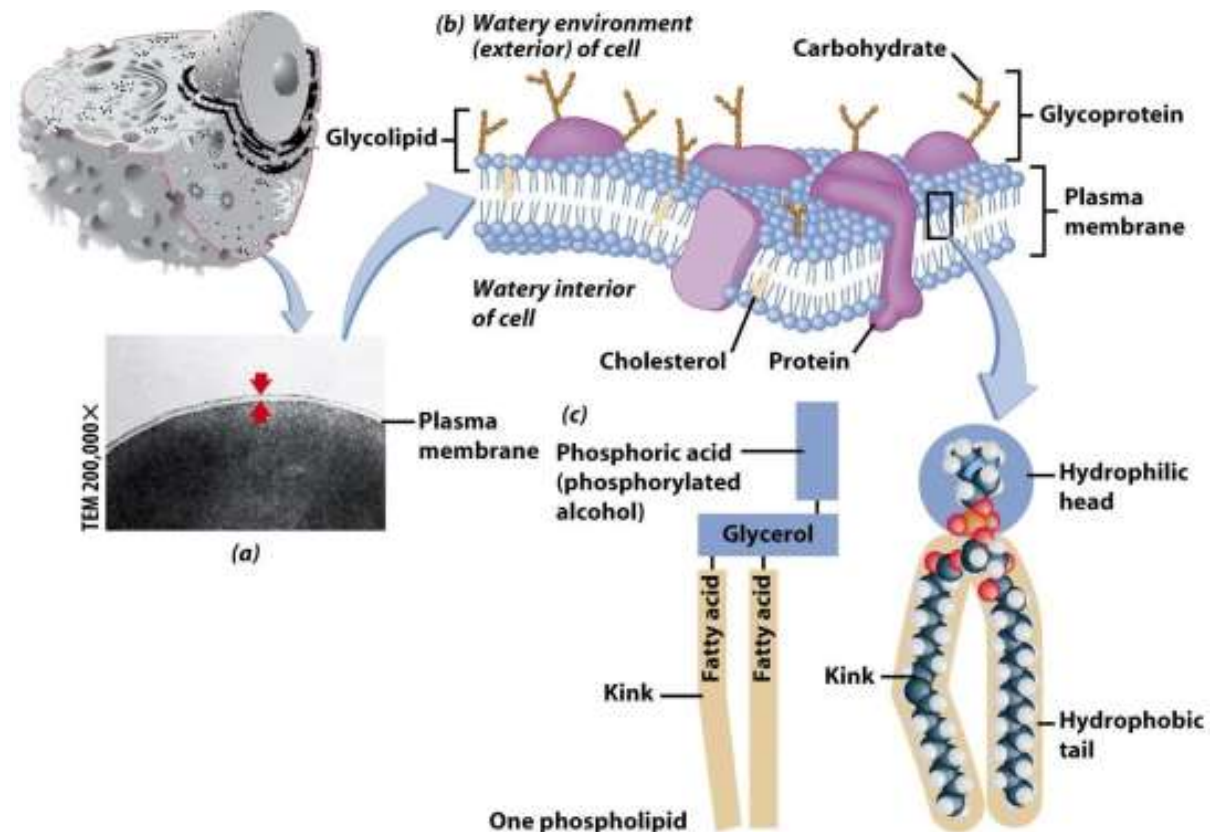


Bacteria-Like Organelles

- Derived from symbiotic bacteria
- Ancient association
- Endosymbiotic theory
 - Evolution of modern cells from cells & symbiotic bacteria

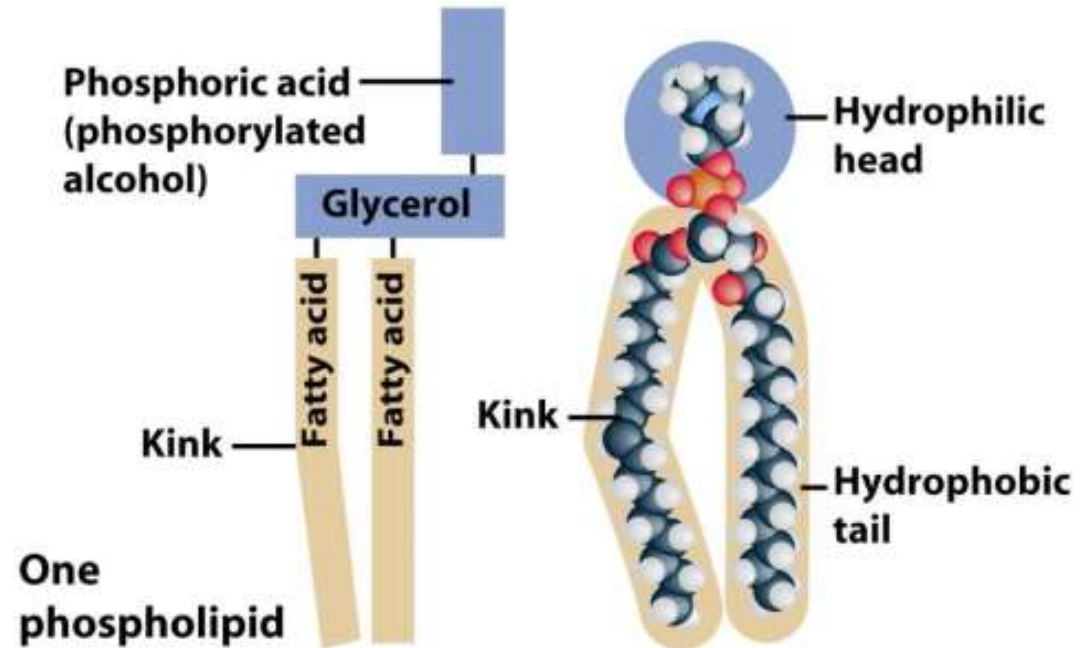
Plasma Membrane

- Contains cell contents
- Double layer of phospholipids & proteins



Phospholipids

- Polar
 - Hydrophylic head
 - Hydrophobic tail
- Interacts with water

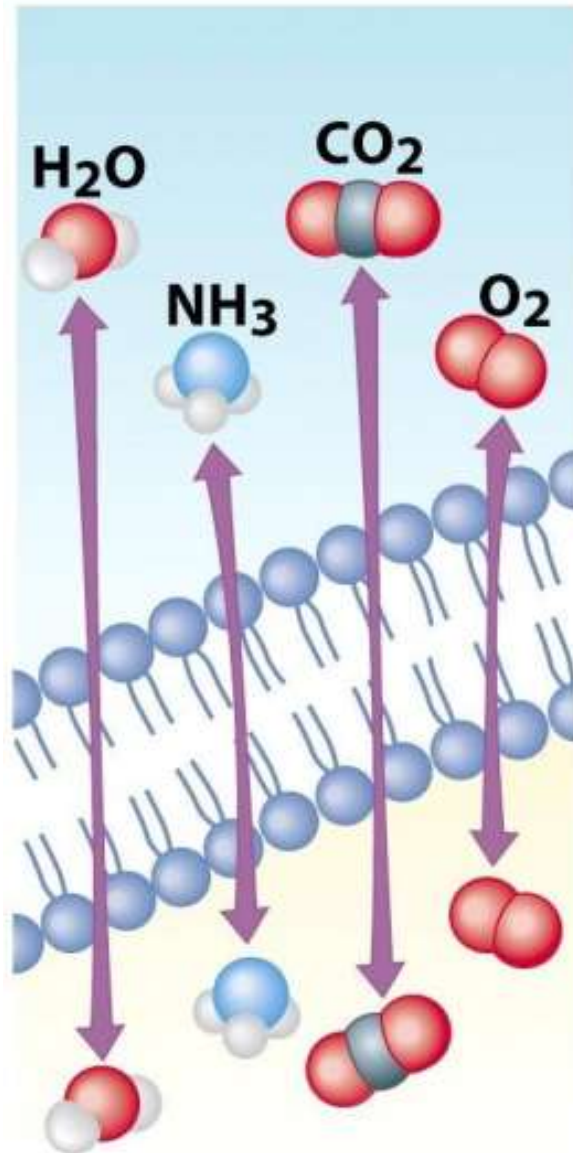


Movement Across the Plasma Membrane

- A few molecules move freely
 - Water, Carbon dioxide, Ammonia, Oxygen
- Carrier proteins transport some molecules
 - Proteins embedded in lipid bilayer
 - Fluid mosaic model – describes fluid nature of a lipid bilayer with proteins

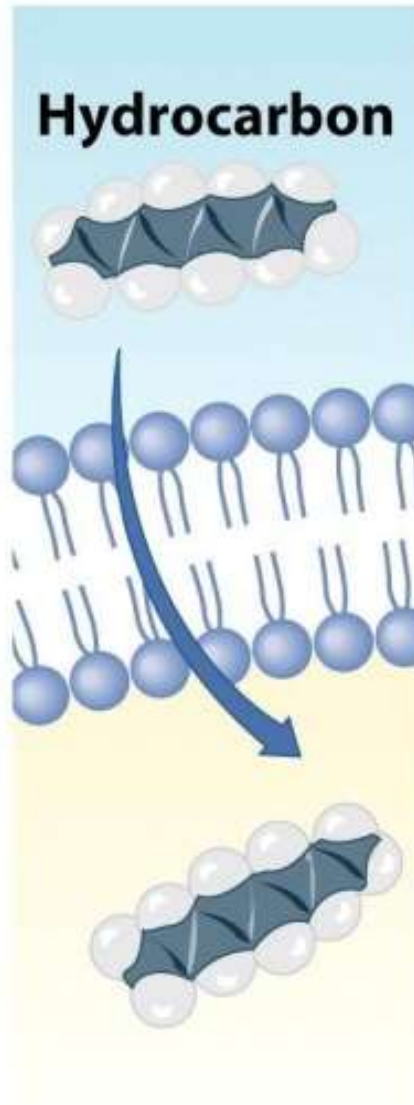
(a)

Small uncharged
molecules



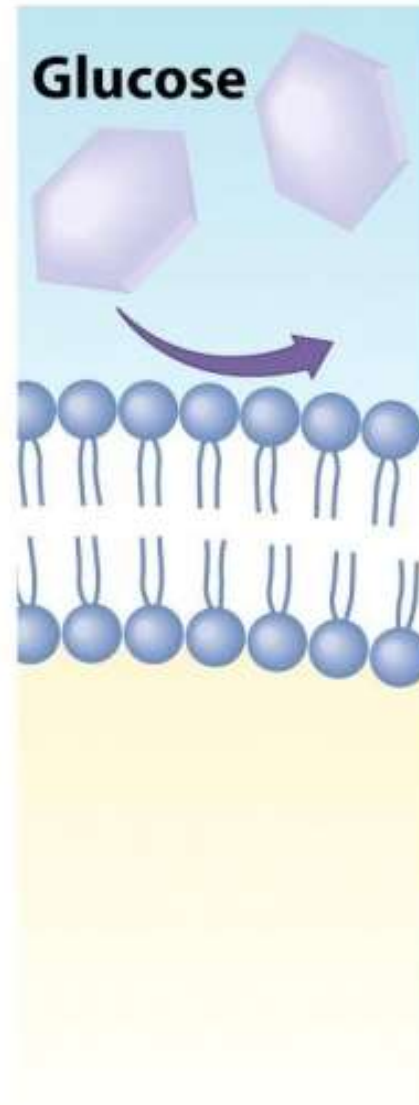
(b)

Lipid-soluble
substances



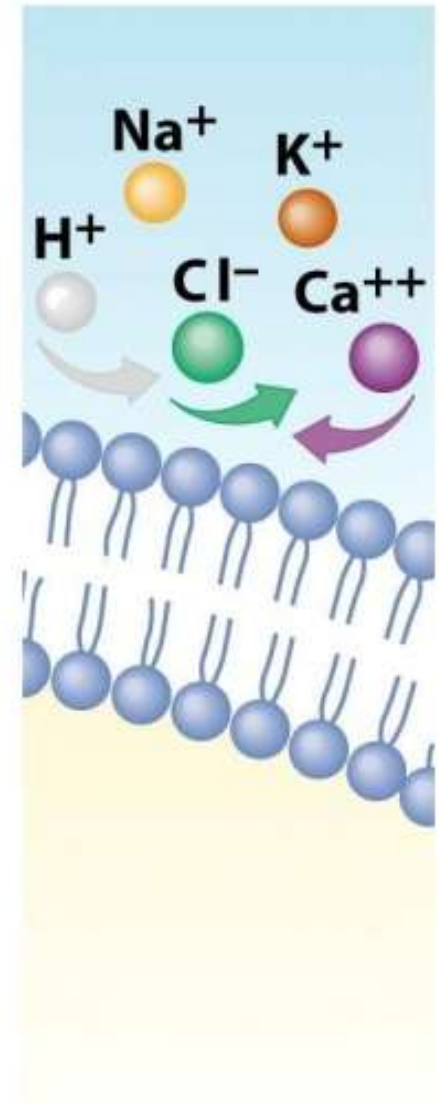
(c)

Water-soluble
substances



(d)

Ions



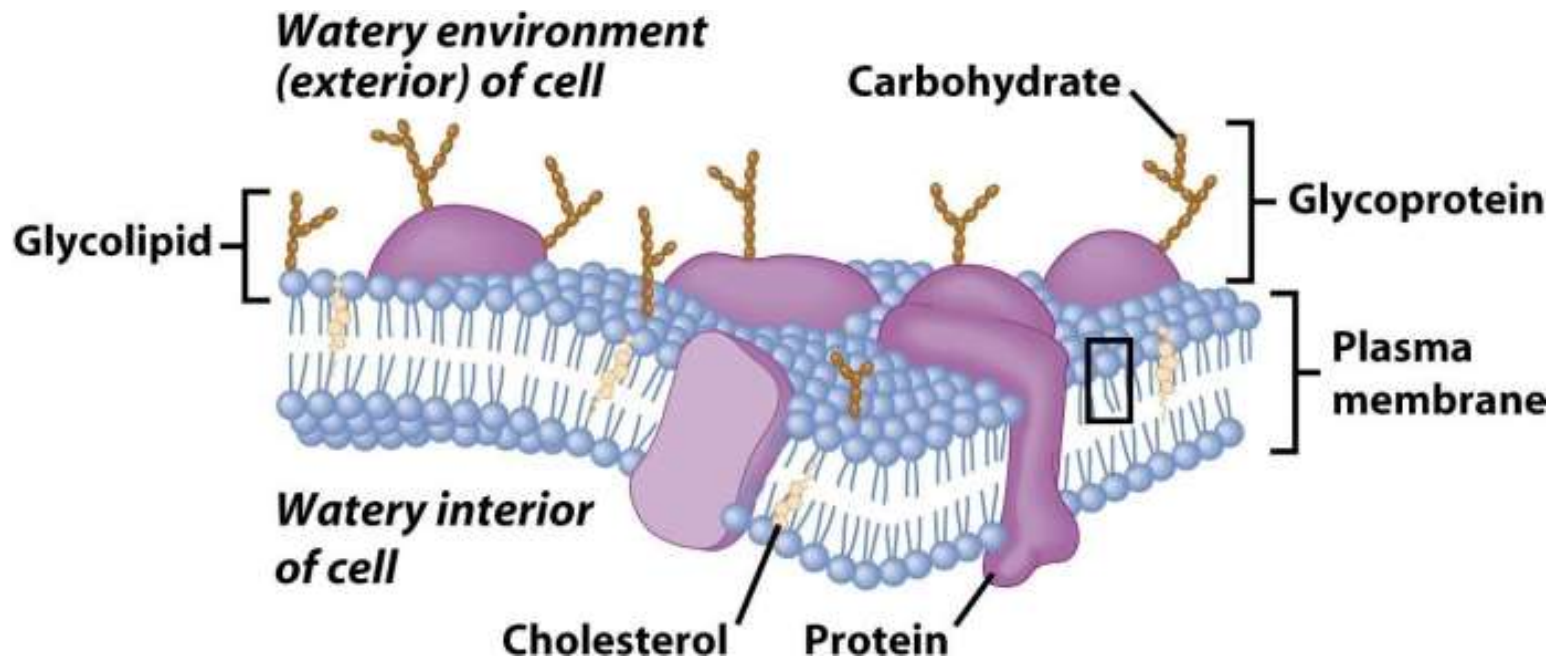
Membrane Proteins

1. Channels or transporters

- Move molecules in one direction

2. Receptors

- Recognize certain chemicals



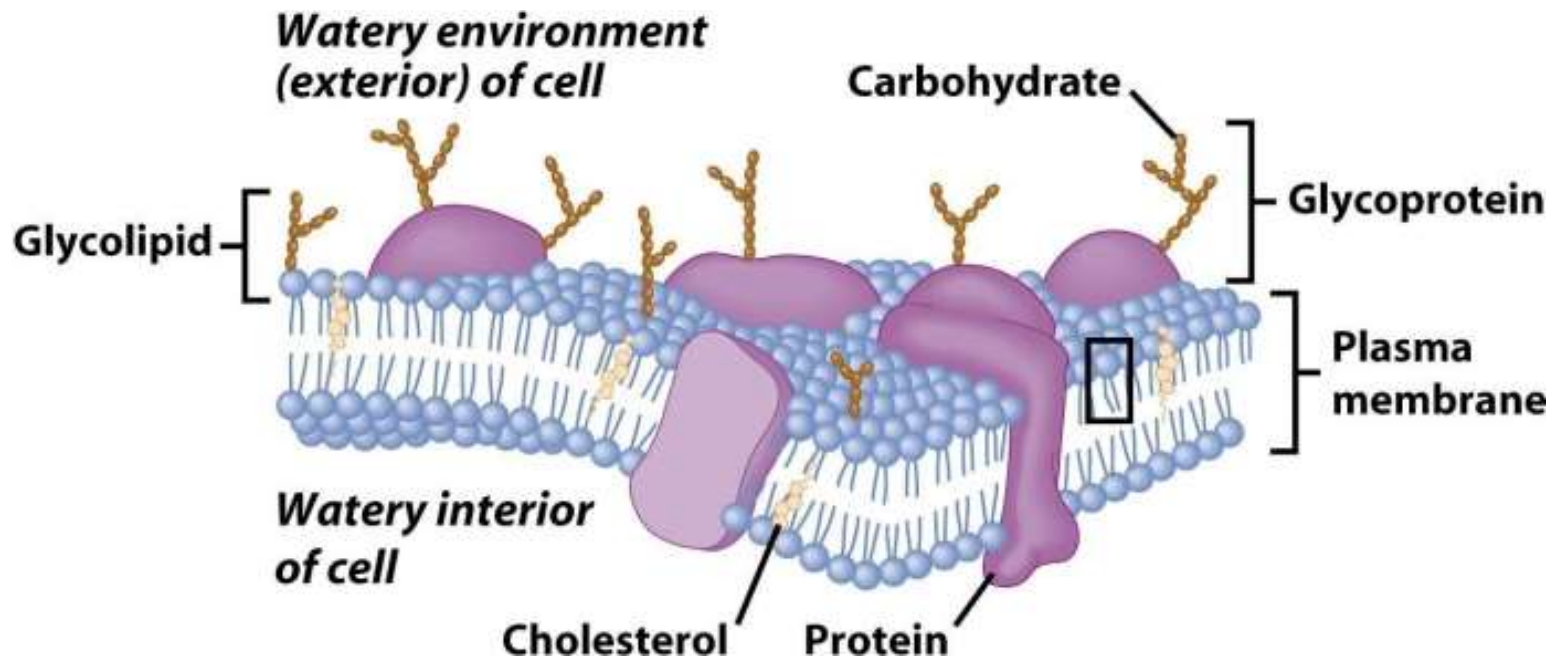
Membrane Proteins

3. Glycoproteins

- Identify cell type

4. Enzymes

- Catalyze production of substances



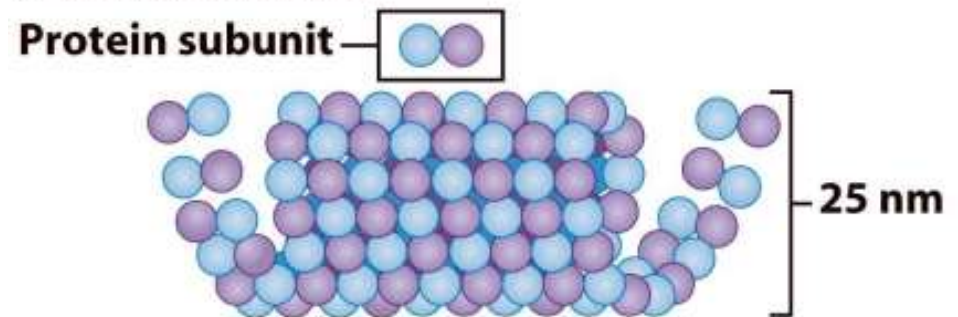
Cytoskeleton

- Filaments & fibers
- Made of 3 fiber types
 - Microfilaments
 - Microtubules
 - Intermediate filaments
- 3 functions:
 - mechanical support
 - anchor organelles
 - help move substances

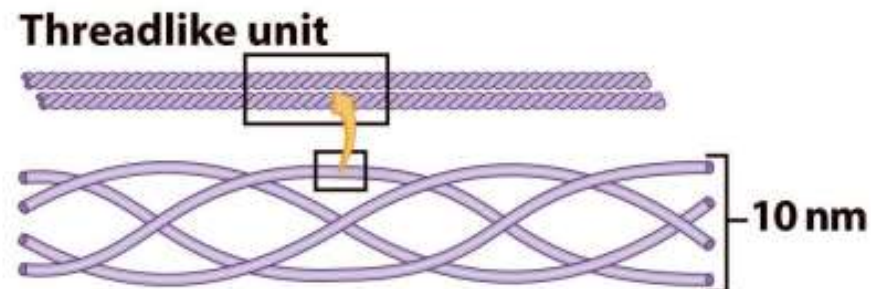
(a) Microfilament



(b) Microtubule



(c) Intermediate filament

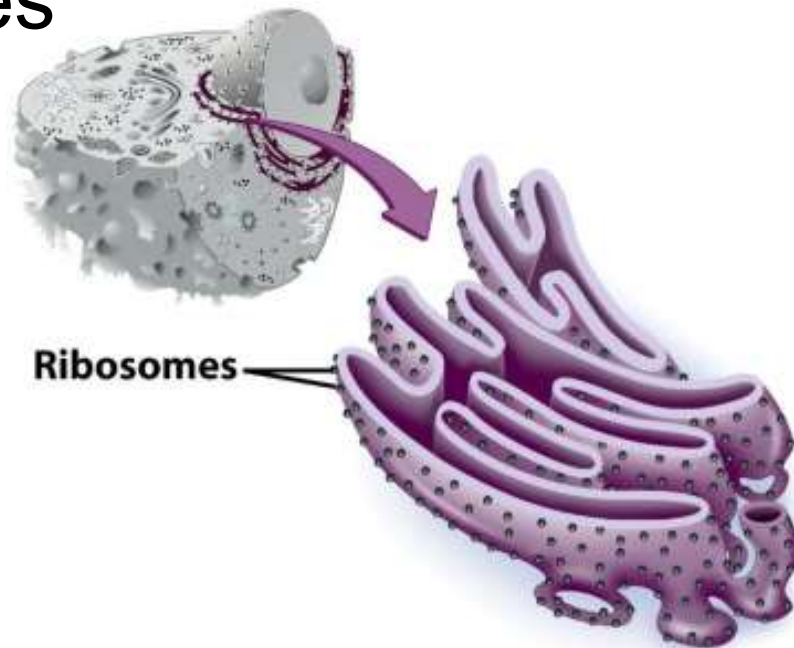


Endoplasmic Reticulum

- Helps move substances within cells
- Network of interconnected membranes
- Two types
 - Rough endoplasmic reticulum
 - Smooth endoplasmic reticulum

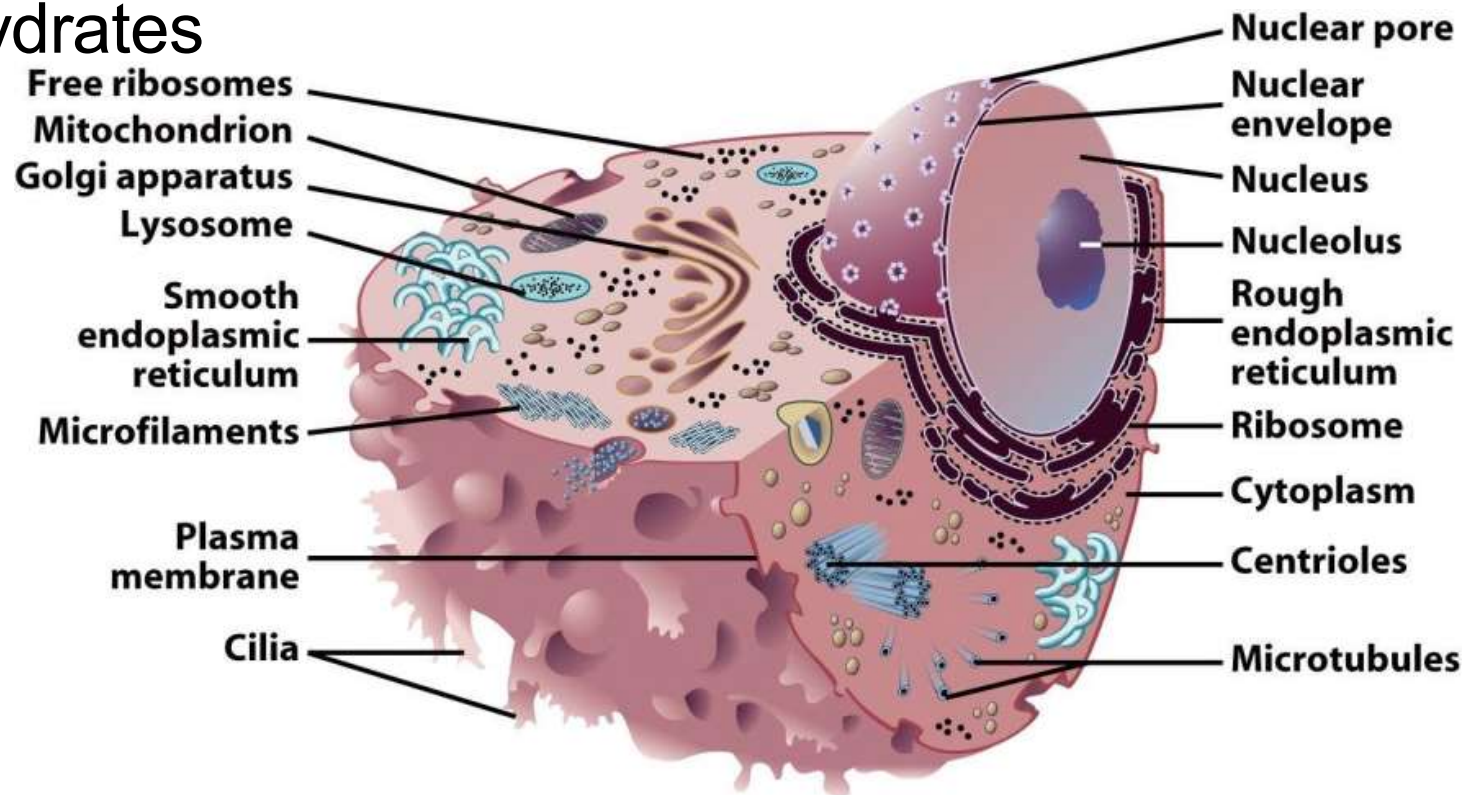
Rough Endoplasmic Reticulum

- Ribosomes attached to surface
 - Manufacture proteins
 - Not all ribosomes attached to rough ER
- May modify proteins from ribosomes



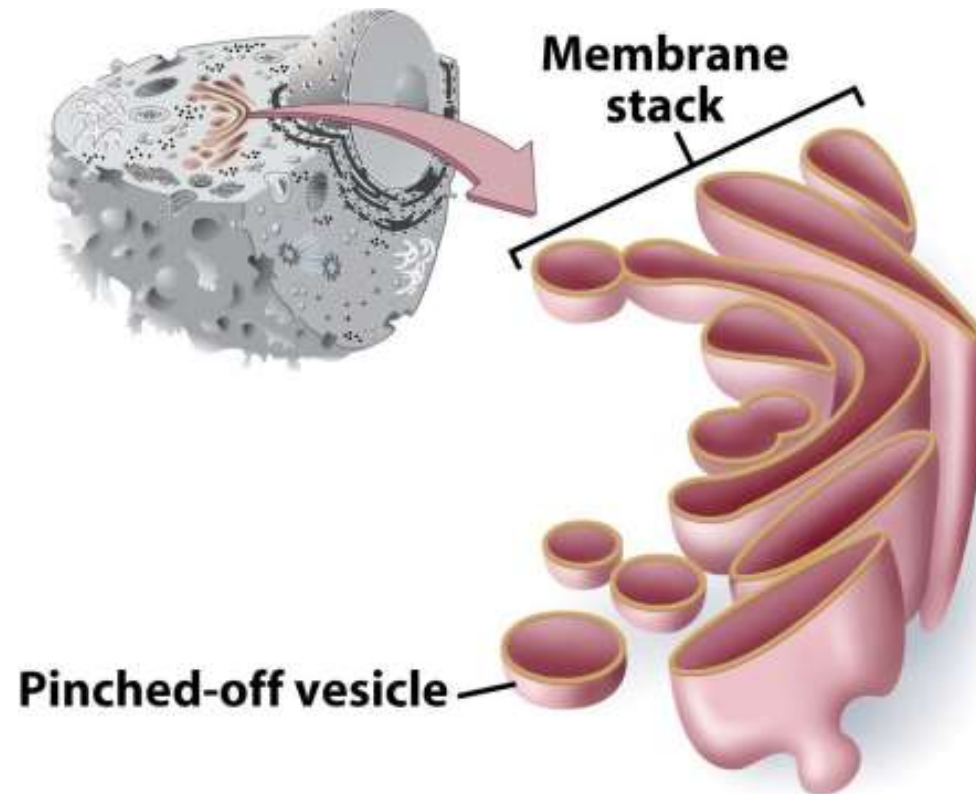
Smooth Endoplasmic Reticulum

- No attached ribosomes
- Has enzymes that help build molecules
 - Carbohydrates
 - Lipids



Golgi Apparatus

- Involved in synthesis of plant cell wall
- Packaging & shipping station of cell



Golgi Apparatus Function

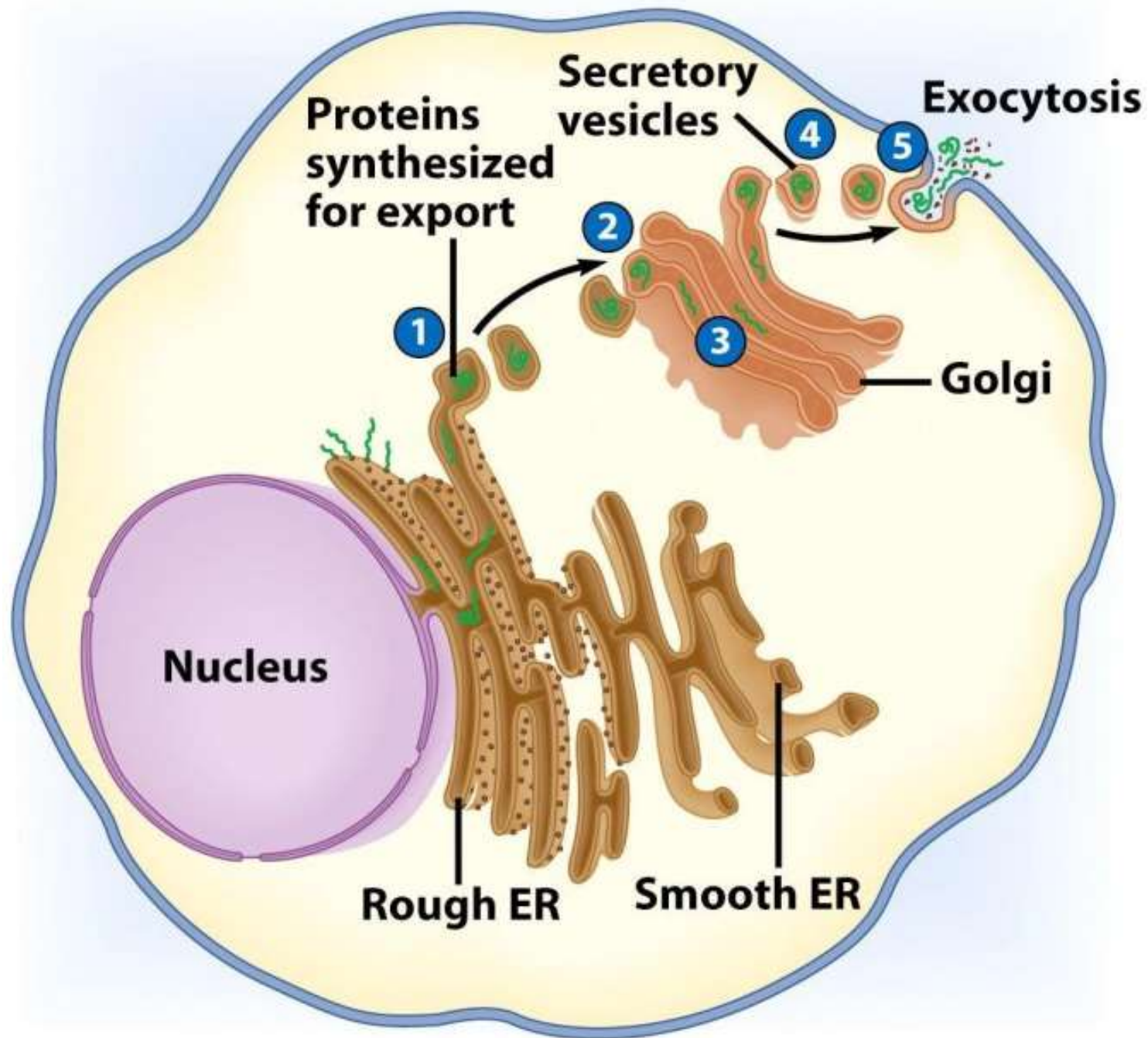
1. Molecules come in vesicles
2. Vesicles fuse with Golgi membrane
3. Molecules may be modified by Golgi

Golgi Apparatus Function (Continued)

4. Molecules pinched-off in separate vesicle

5. Vesicle leaves Golgi apparatus

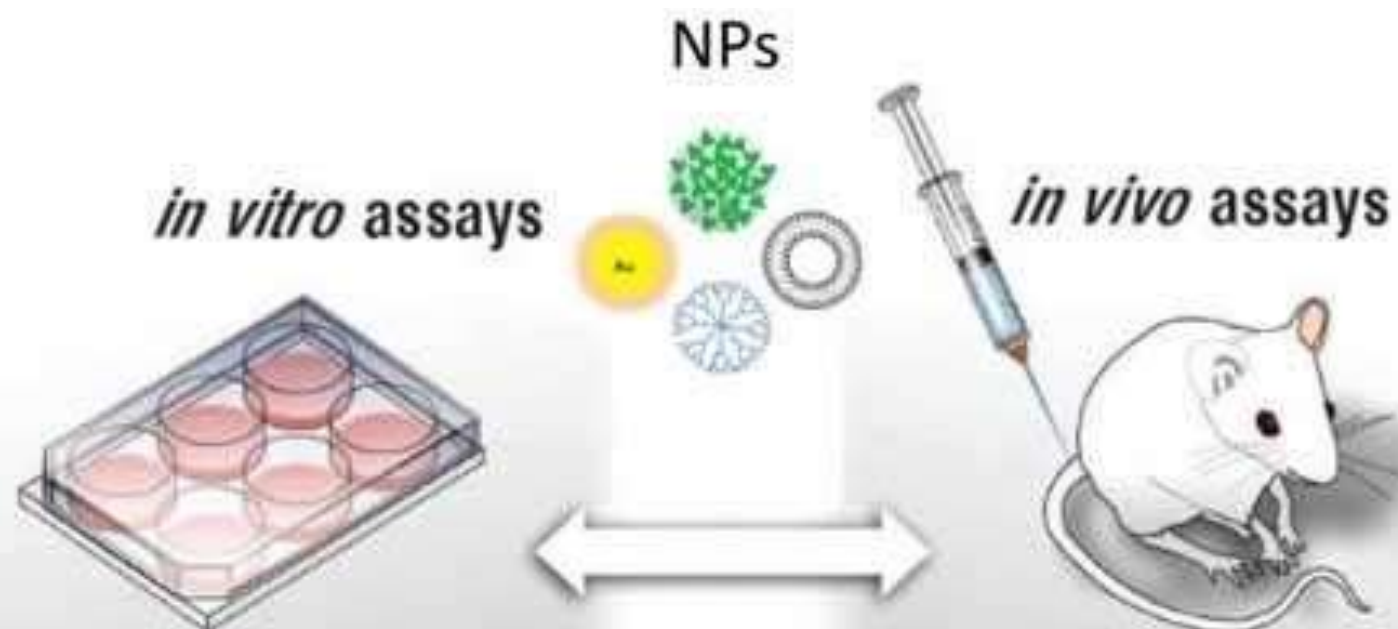
6. Vesicles may combine with plasma membrane to secrete contents



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 already established.

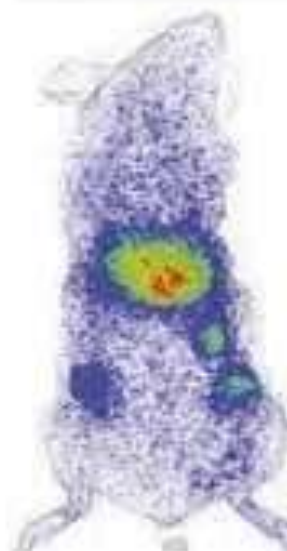
- Cells, removed from animal tissue or whole animals, will continue to grow if supplied with nutrients and growth factors.
- This process is called cell culture. It occurs in vitro ('in glass') as opposed to in vivo ('in life').



Transformed cell lines



Primary cells



Biodistribution

Mouse	<i>D. melanogaster</i>
Rat	<i>C. elegans</i>
Rabbit	<i>C. intestinalis</i>
Guinea pig	<i>P. guthriei</i>
Pig/piglet	<i>E. foecalis</i>
Marmoset	<i>P. lewisii</i>
	<i>M. galapagensis</i>
	<i>D. rerio</i>

Alternative models

- The culture process allows single cells to act as independent units much like any microorganism such as bacteria or fungi.
- The cells are capable of division by mitosis and the cell population can continue growth until limited by some parameter such as nutrient depletion.

WHAT IS CELL CULTURE USED FOR?

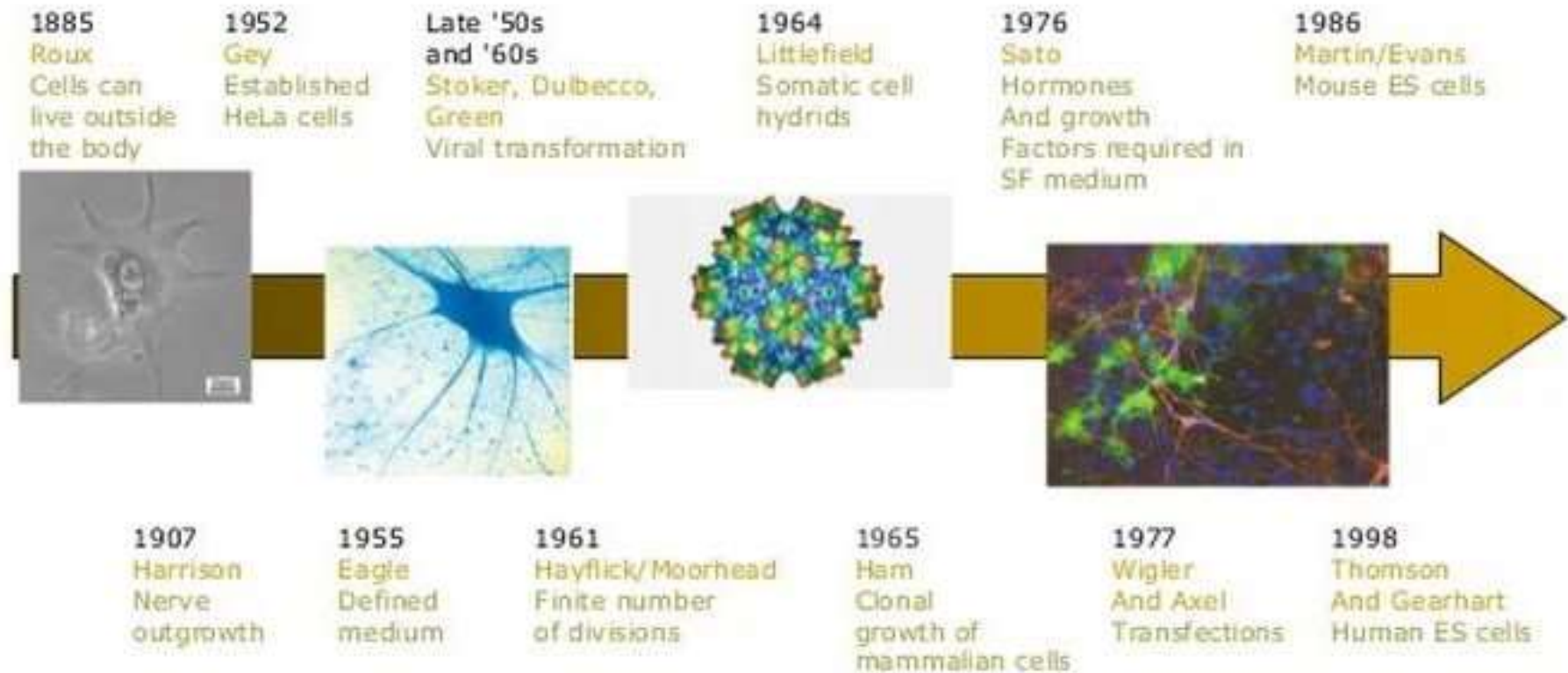
Areas where cell culture technology is currently playing a major role:-

- 1) **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
- 2) **Toxicity testing** : Study the effects of new drugs
- 3) **Cancer research** : Study the function of various chemicals, virus & radiation to convert normal cultured cells to cancerous cells
- 4) **Virology** : Cultivation of virus for vaccine production, also used to study their infectious cycle.
- 5) **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
- 6) **Gene therapy** : Cells having a functional gene can be replaced to cells which are having non-functional gene

Cell Culture *in vitro*- A brief history

- 1885: Roux maintained embryonic chick cells alive in saline solution for short lengths of time.
- 1912: Alexis Carrel cultured connective tissue and showed heart muscle tissue contractility over 2-3 months.
- 1943: Earle *et al.* produced continuous rat cell line.
- 1962: Buonassisi *et al.* Published methods for maintaining differentiated cells of tumour origin.
- 1970: Gordon Sato *et al.* published the specific growth factor and media requirements for many cell types.
- 1979: Bottenstein and Sato defined a serum-free medium for neural cells.
- 1980: Chinese Hamster Ovary (CHO) cell lines were developed. Recombinant erythropoietin was produced on CHO cell by AMGEN (USA).

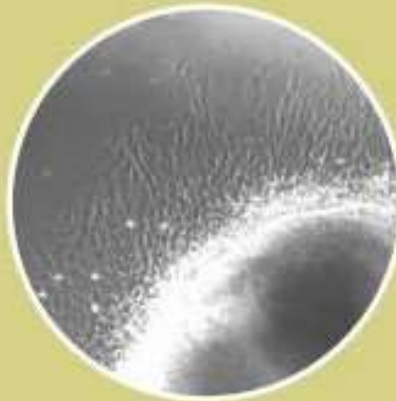
[History of cell culture]



Establishment of Tissue Culture



Organ
Culture



Primary
Explant



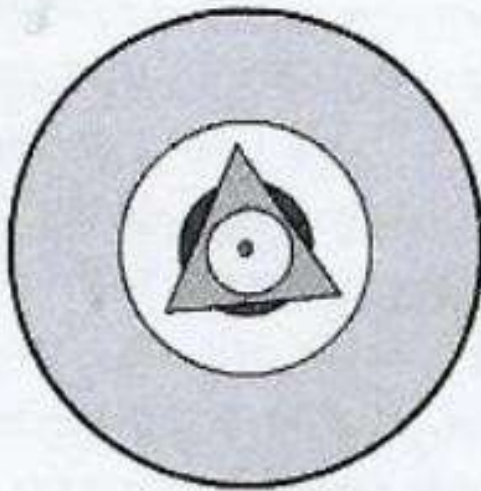
Cell
Culture



Tissue Culture:

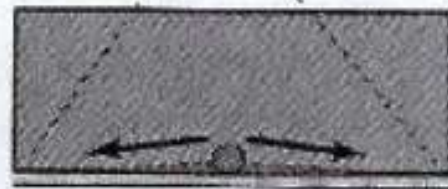
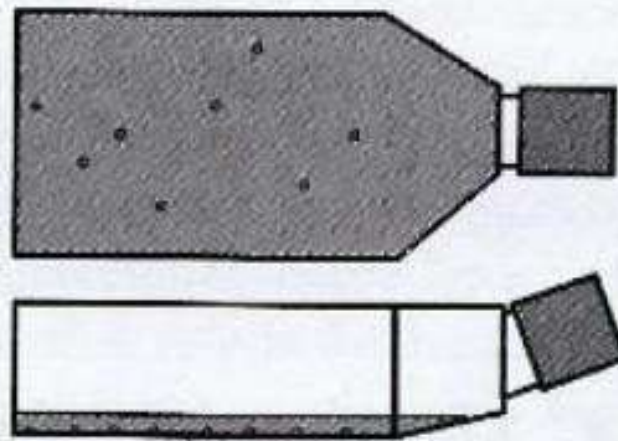
- ❖ Organ culture: tissue is cultured at the liquid-gas interphase (on raft, gel) as to retain the spherical/3D shape of the tissue.
- ❖ Primary explant culture: tissue fragment is placed at glass/plastic-liquid interface; cell migrates in the plane and form outgrowth after attachment.
- ❖ Cell culture: explant/outgrowth from the primary explant is dispersed (mechanically/enzymatically) into a cell suspension that can be cultured as an adherent monolayer.

ORGAN CULTURE



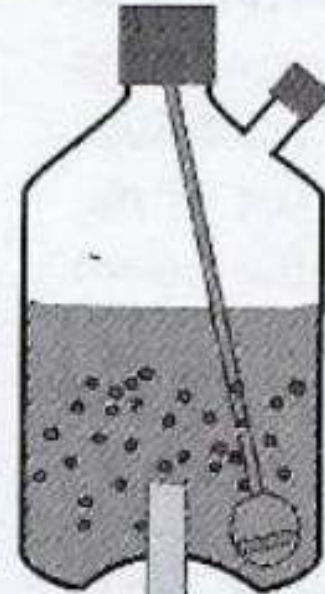
Tissue at gas-liquid interface; histological structure maintained

EXPLANT CULTURE



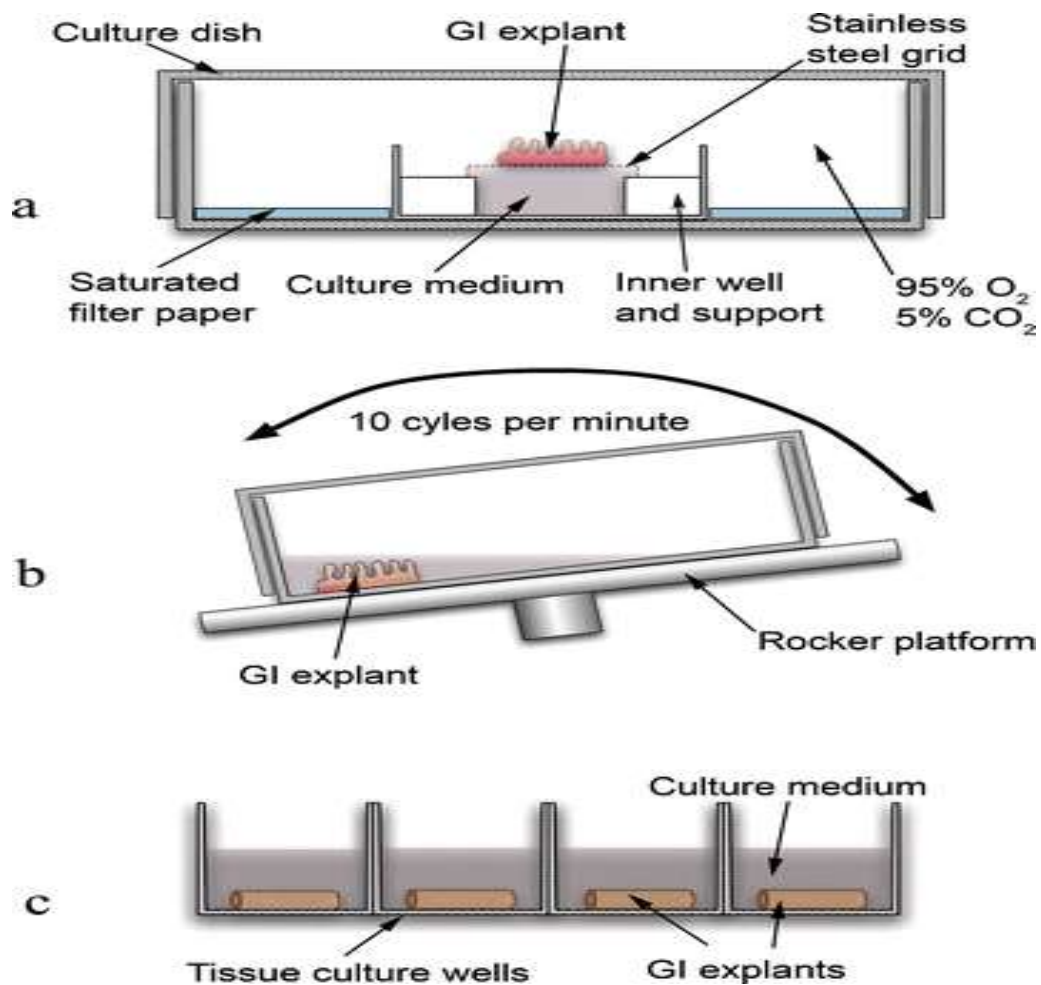
Tissue at solid-liquid interface; cells migrate to form outgrowth

DISSOCIATED CELL CULTURE

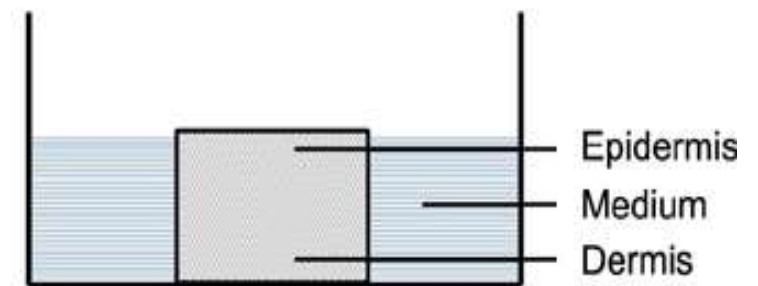


Disaggregated tissue; cells form monolayer at solid-liquid interface

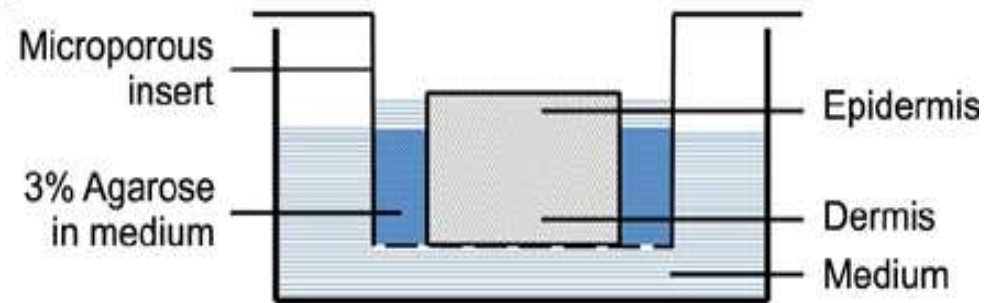
Organ Culture



A

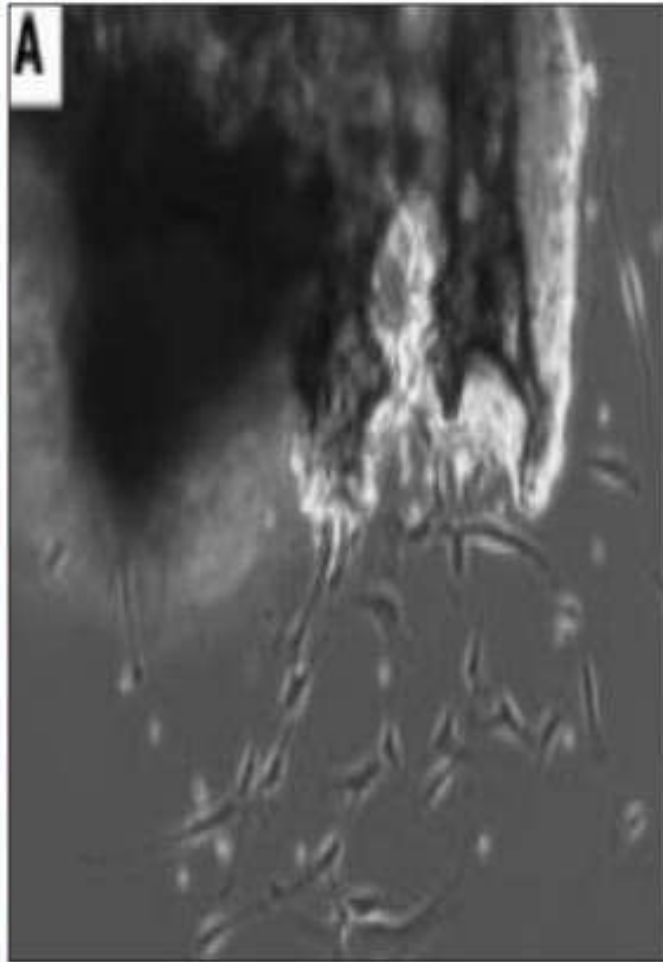


B





Human skin in medium



Primary explant from skin
tissue showing the cell
outgrowth



Cell culture of SK-MEL28

Classes of Culture Cells

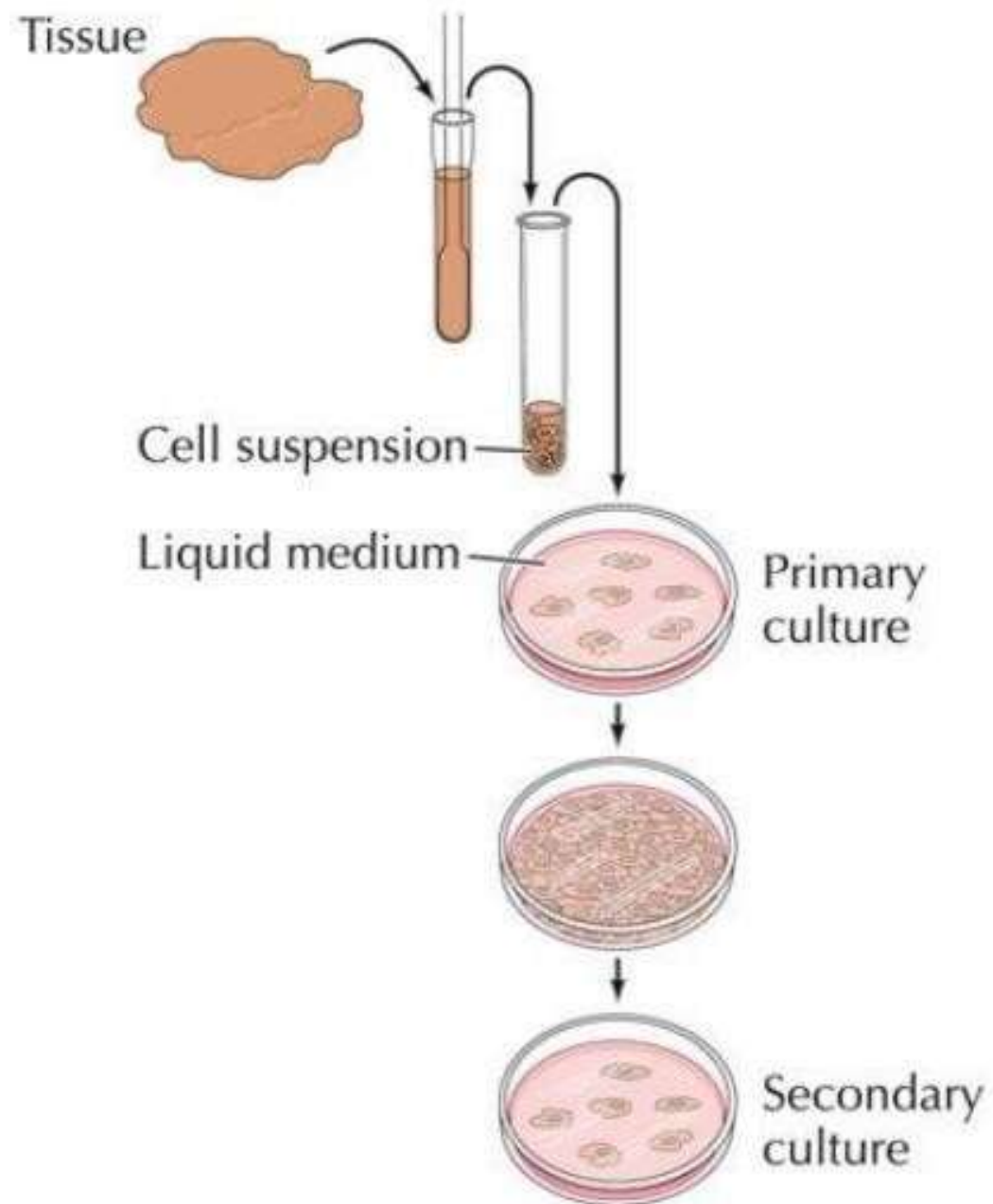
- Cultures of animal cells are usually divided into 3 classes:
 1. Primary cells
 2. Cell lines
 3. Continuous cell lines

Primary Culture

- Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (i.e., reach confluence).
- At this stage, the cells have to be subcultured (i.e., passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

Cell Line

- After the first subculture, the primary culture becomes known as a cell line or subclone or secondary culture.
- Cell lines derived from primary cultures have a limited life span and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.



Finite Cell Line

- Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as senescence; these cell lines are known as finite.

Continuous Cell Line

- However, some cell lines become immortal through a process called **transformation**, which can occur spontaneously or can be chemically or virally induced.
- When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line.

Difference between finite and continuous cell line

PROPERTY	FINITE CELL LINE	CONTINUOUS CELL LINE
GROWTH RATE	SLOW	FAST
Mode of growth	monolayer	Suspension or monolayer
yield	low	High
transformation	normal	Immortal, tumorigenic
Anchorage dependence	yes	No
Contact inhibition	yes	no
Cloning efficiency	Low	High
Serum requirement	High	High
Markers	Tissue specific	Chromosomal, antigenic or enzymatic

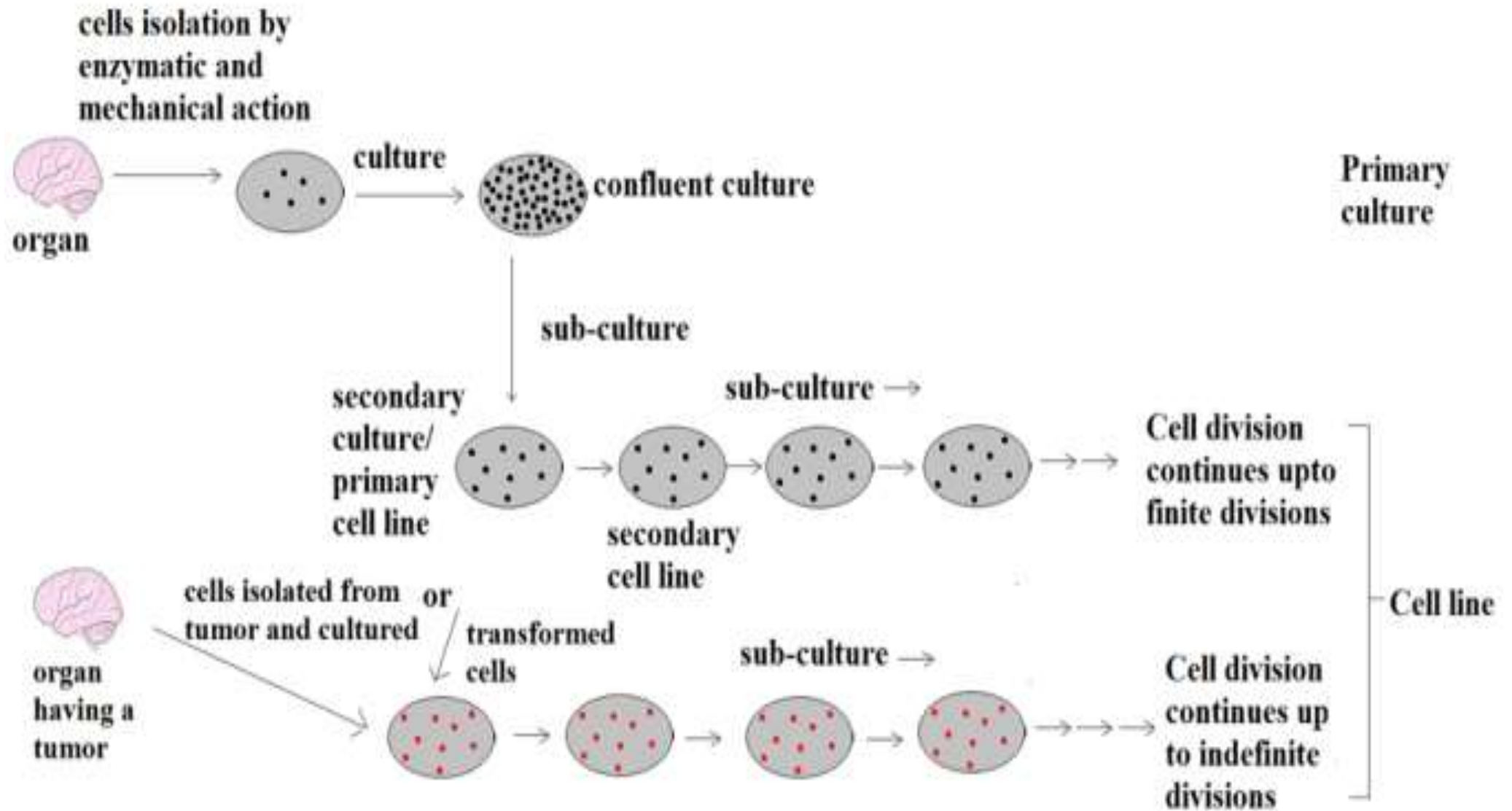


fig: animal cell culture

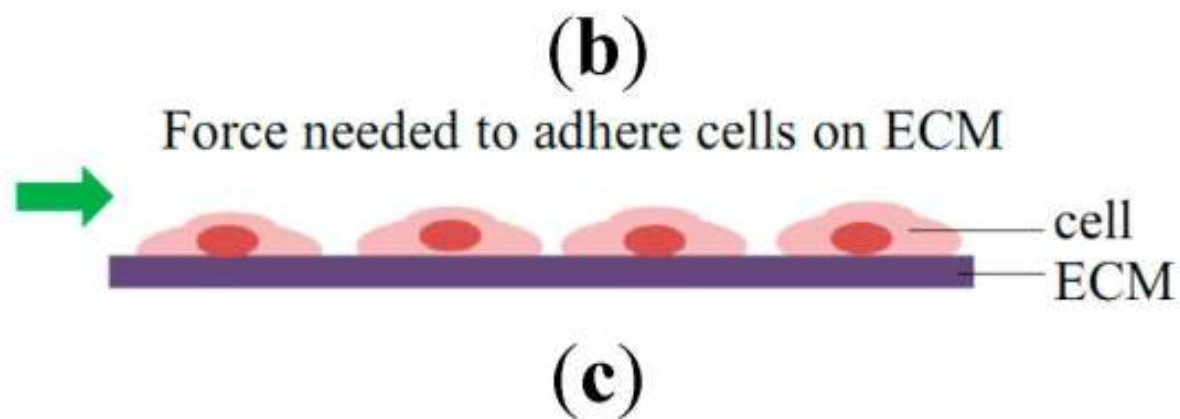
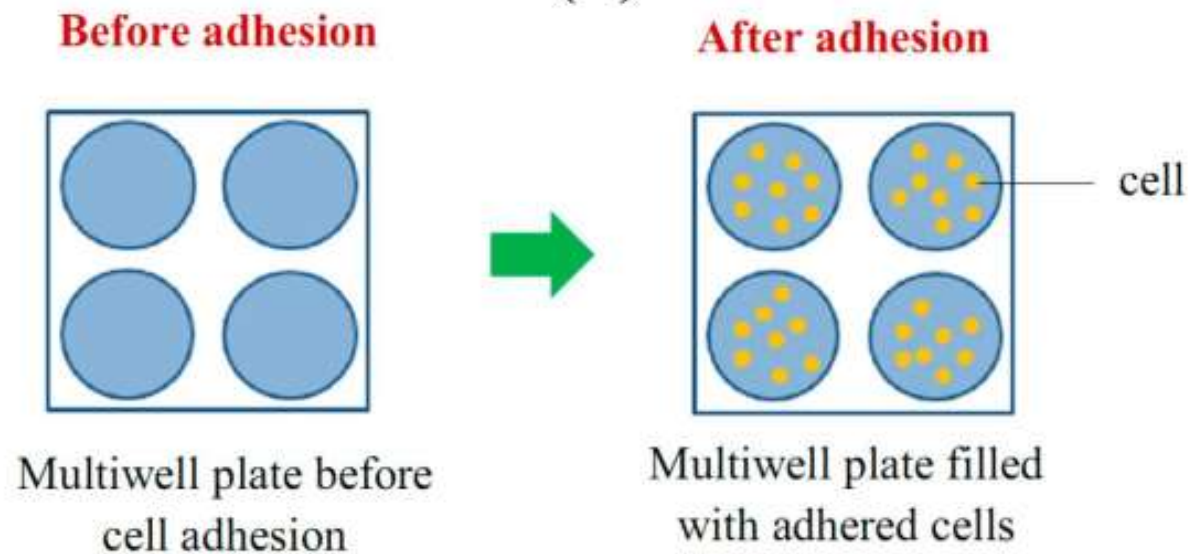
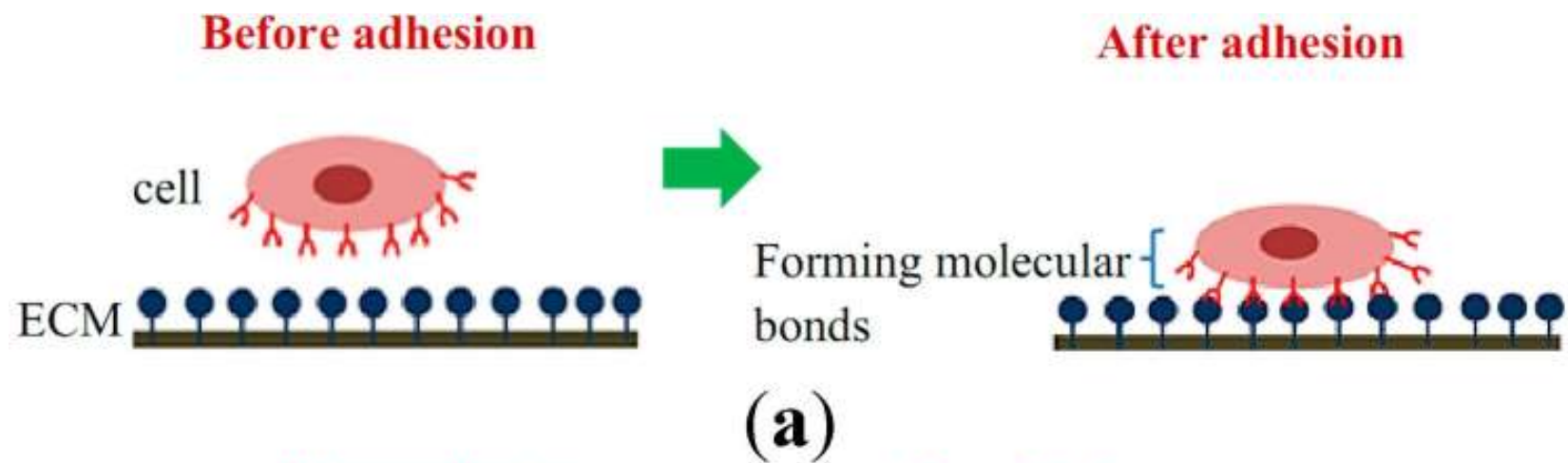
- Depending on their origin, primary cells grow in two different ways:

1. Adherent monolayer cells

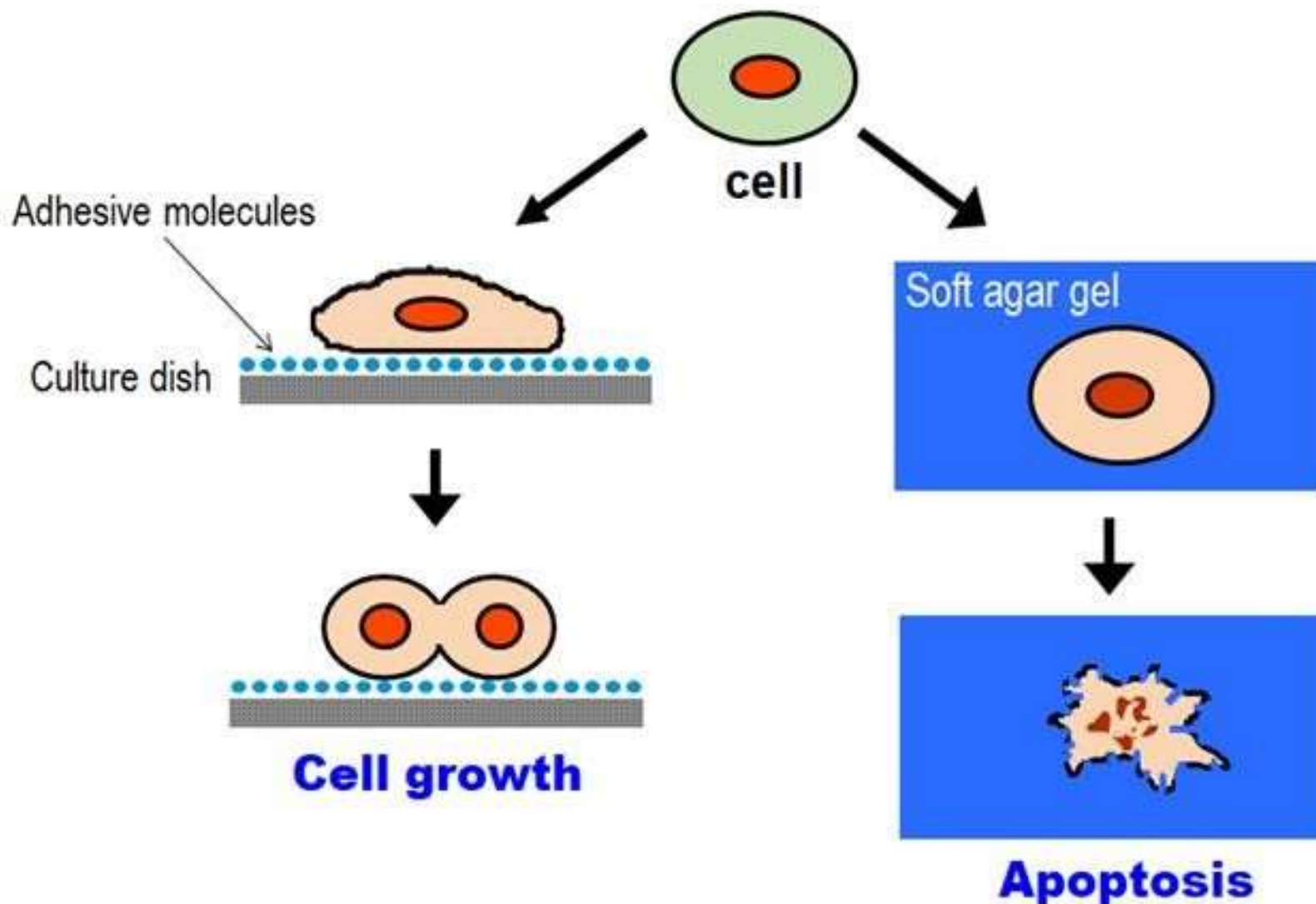
2. Suspension cells

Adherent cells

- These cells are anchorage dependent and propagate as a monolayer.
- These cells need to be attached to a solid or semi-solid substrate for proliferation.
- These adhere to the culture vessel with the use of an extracellular matrix which is generally derived from tissues of organs that are immobile and embedded in a network of connective tissue.
- Fibroblasts and epithelial cells are of such types.



Cells cannot secure their anchorage in soft agar and die by themselves through activating apoptotic signaling pathway



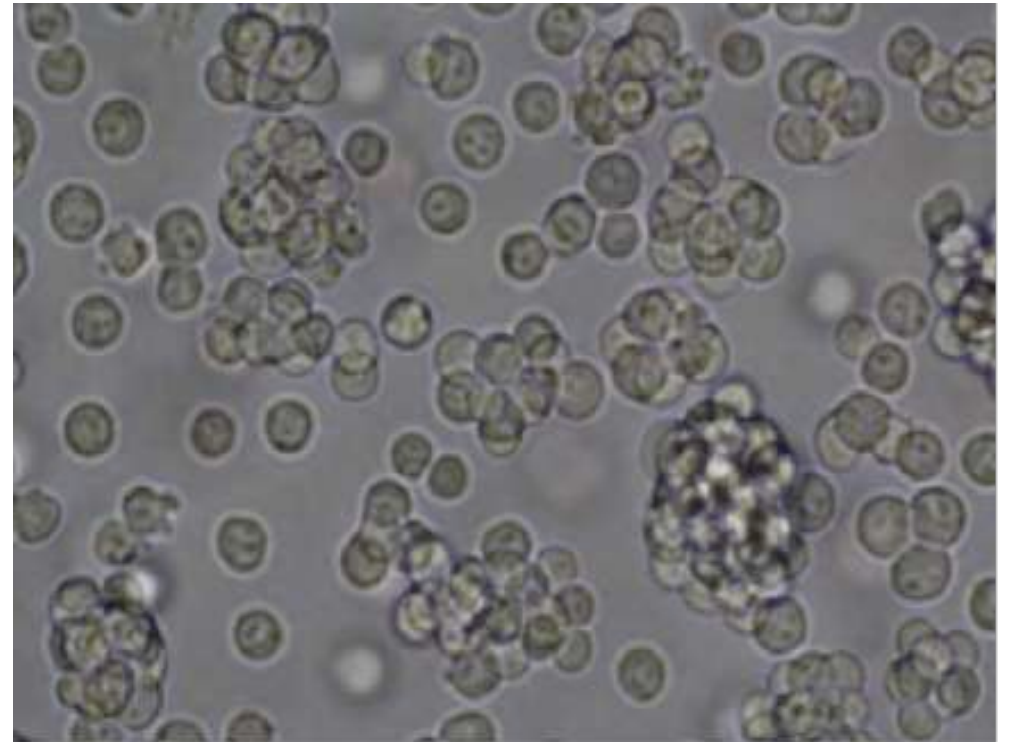
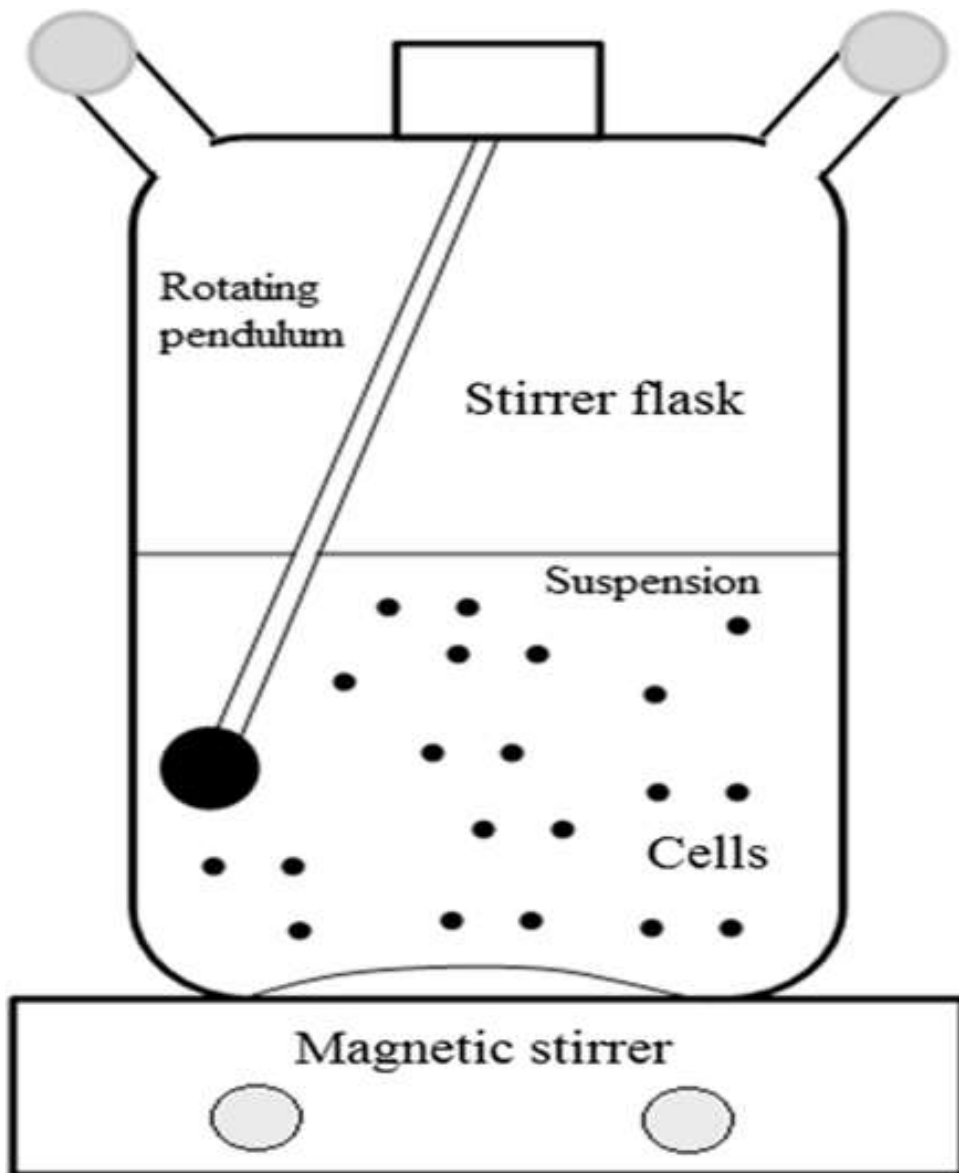
Suspension cells

- Suspension cells do not attach to the surface of the culture vessels.
- These cells are also called anchorage independent or non-adherent cells which can be grown floating in the culture medium.
- Hematopoietic stem cells (derived from blood, spleen and bone marrow) and tumor cells can be grown in suspension.

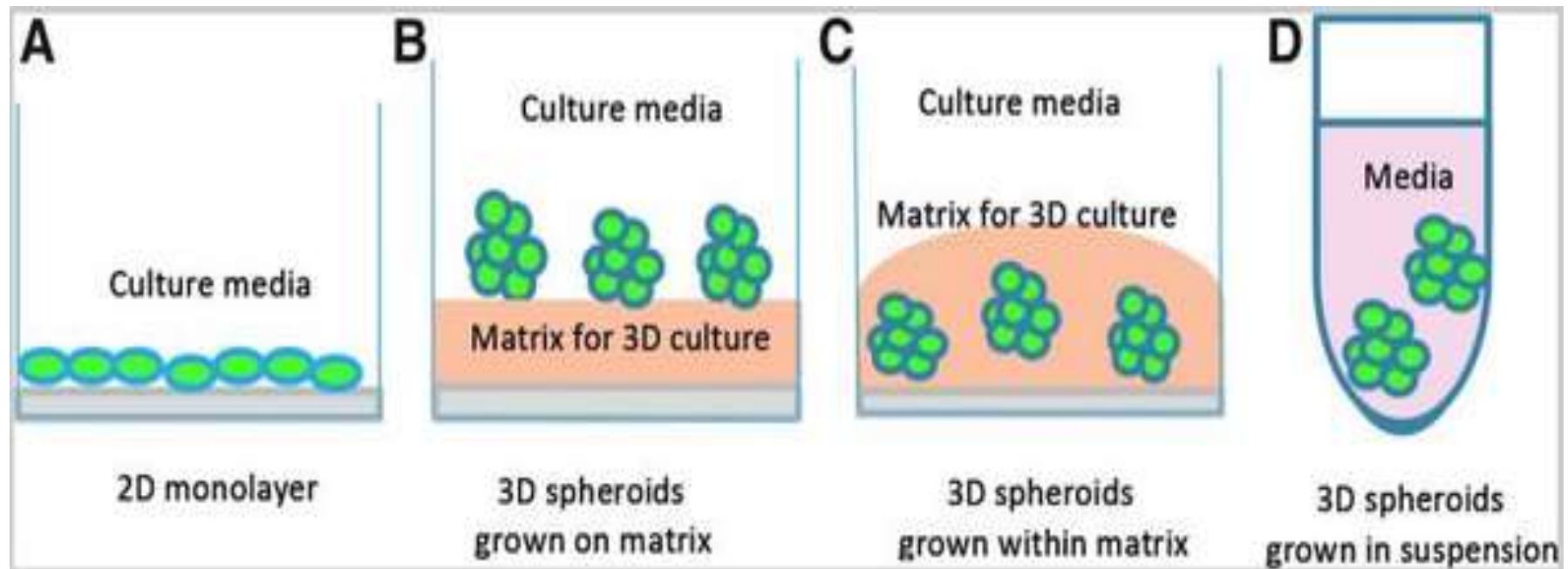
Suspension Cells

- These cells grow much faster which do not require the frequent replacement of the medium and can be easily maintained.
- These are of homogeneous types and enzyme treatment is not required for the dissociation of cells; similarly these cultures have short lag period.

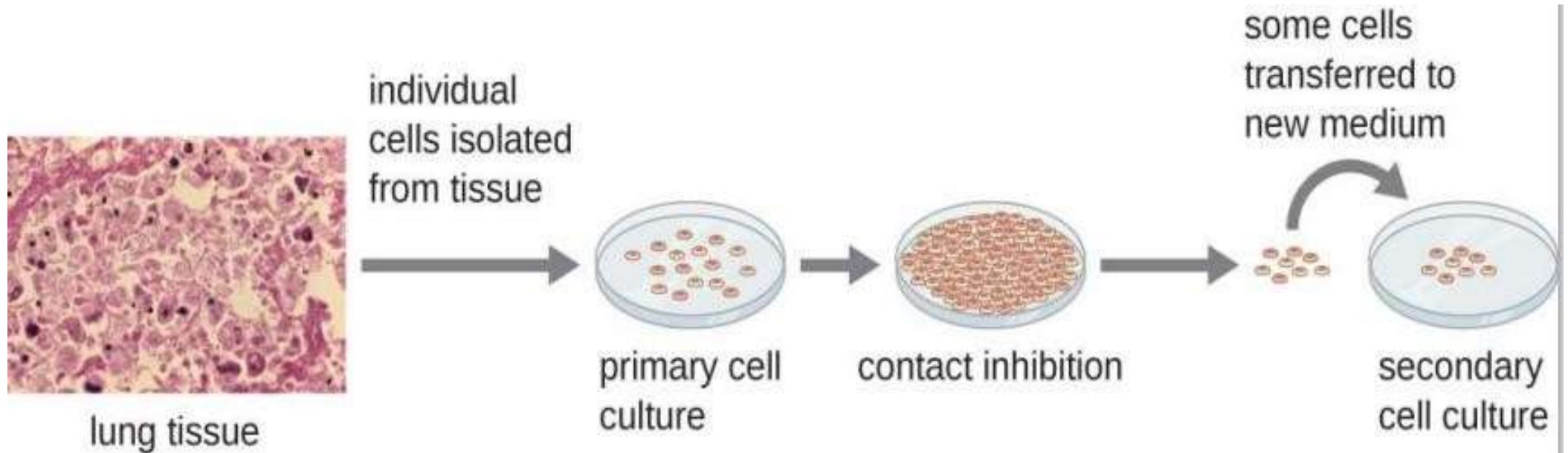
Suspension Cells



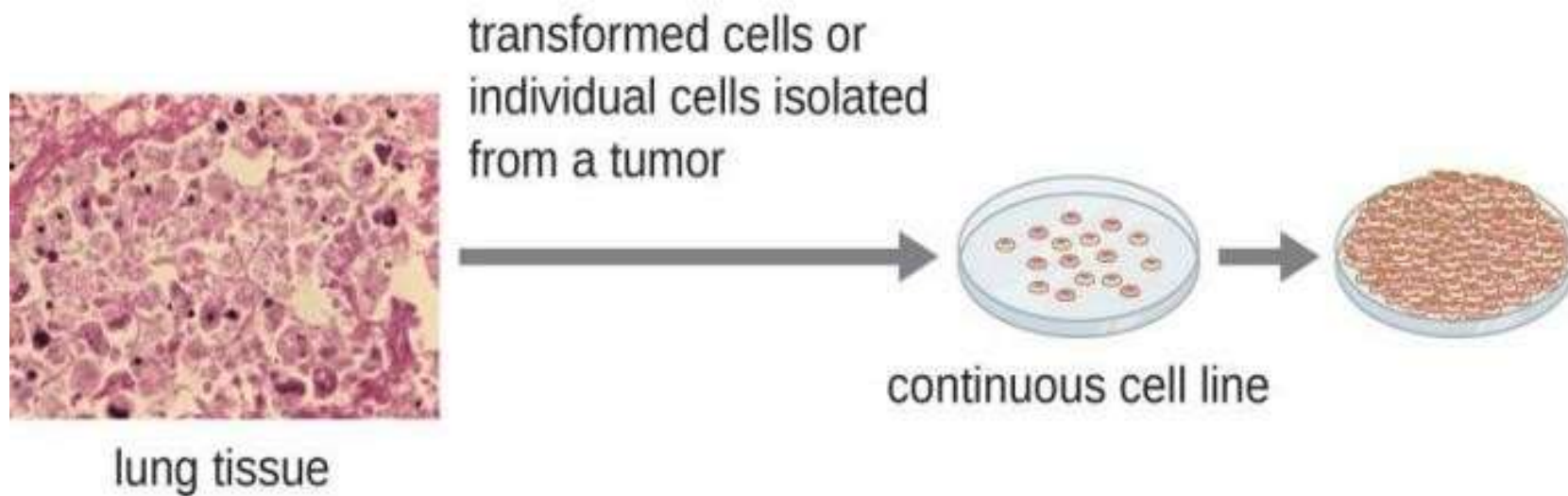
Monolayer Cells vs Suspension Cells



Contact Inhibition



(a)



Demonstrating Contact Inhibition



Normal cells in dish



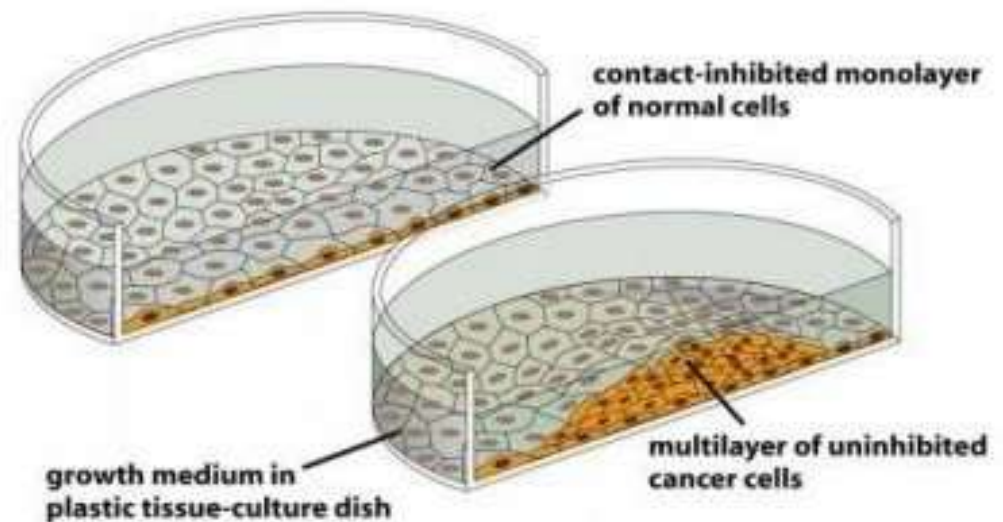
When cells are removed, cells at the edge of gap begin to divide



Normal cells stop dividing when bottom of dish is covered.



Cancer cells continue to divide, piling on top of each other



COMMON CELL LINES

Human cell lines

- ⊙ MCF-7 : breast cancer
- ⊙ HeLa : Henrietta lacks cells

Mammalian cell lines

- ⊙ Vero: African green monkey kidney (epithelial cells)
- ⊙ BHK: Baby Hamster Kidney cells (fibroblast cells)
- ⊙ MDCK: Mardin Darby Chicken Kidney cells

Insect cell lines

- ⊙ C636: *Aedes albopictus* (mosquito cells line)

Morphology of Cells in Culture

❖ Based on **shape** and **appearance**: **3 types**

- Fibroblast-like
- Epithelial-like
- Lymphoblast-like

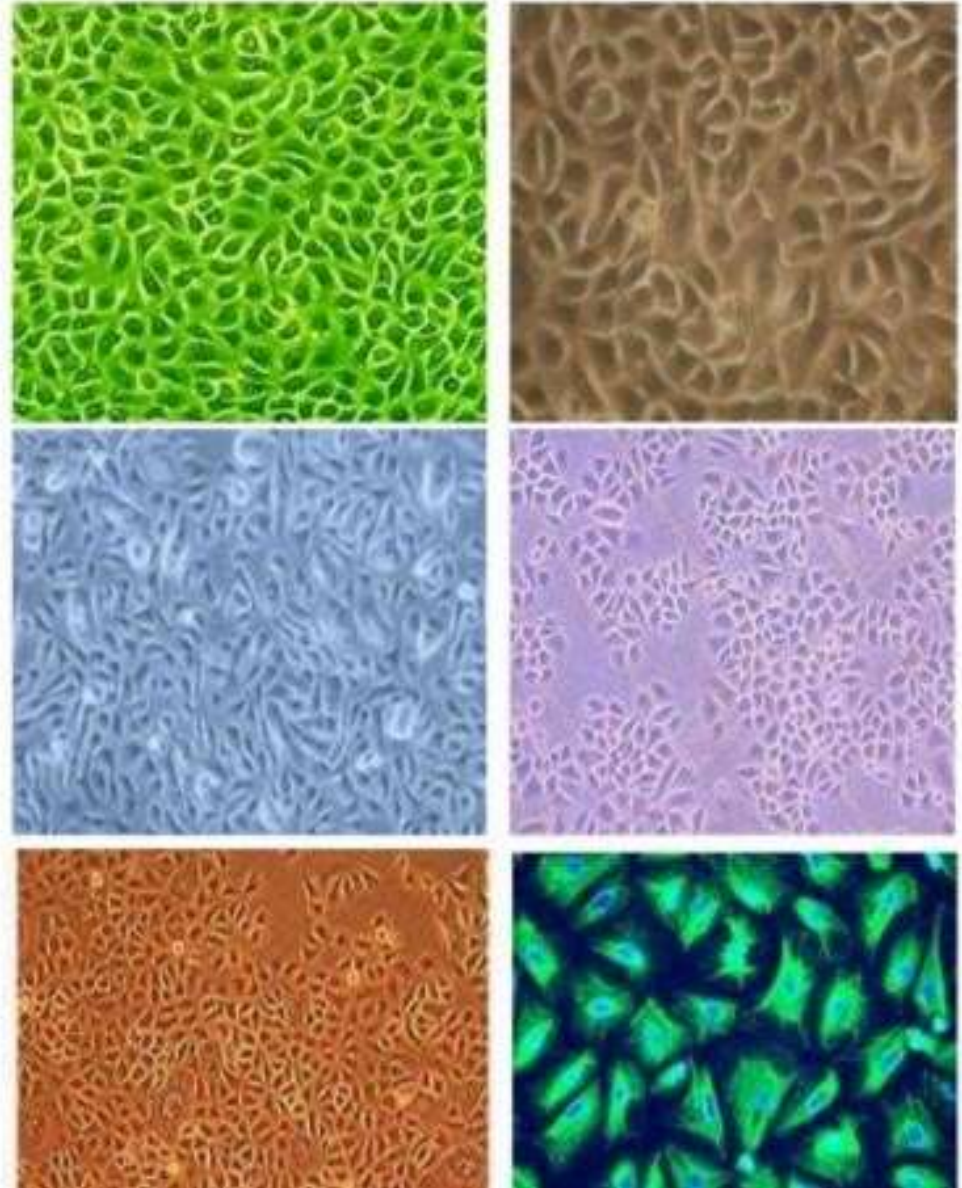
Morphology of Cells in Culture

- **Fibroblast-like** cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate



Morphology of Cells in Culture

• **Epithelial-like** cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.



Primary cells differ from cell lines in many ways

- Primary cells have a finite lifespan. After a certain period of time in culture, they will die. And the amount of time primary cells survives in culture varies depending on the cell type. On the contrary, an immortalized or continuous cell line has acquired the ability to proliferate indefinitely.
- Primary cell cultures are harsh, requiring optimized growth conditions, including the addition of specific cytokines and growth factors for propagation in serum-free or low-serum growth media, which is absolutely different from immortalized cell lines.

Primary cells differ from cell lines in many ways

- Primary cells retain the natural feature of the tissue where they had been isolated. And due to this character, they are physiologically normal and thus theoretically provide unaltered, “natural” experimental results.
- Immortalized cell lines, on the other hand, are often transformed with other inducible modifications, which may alter the outcome of experiments.

The list of major differences between primary cells and cell lines.

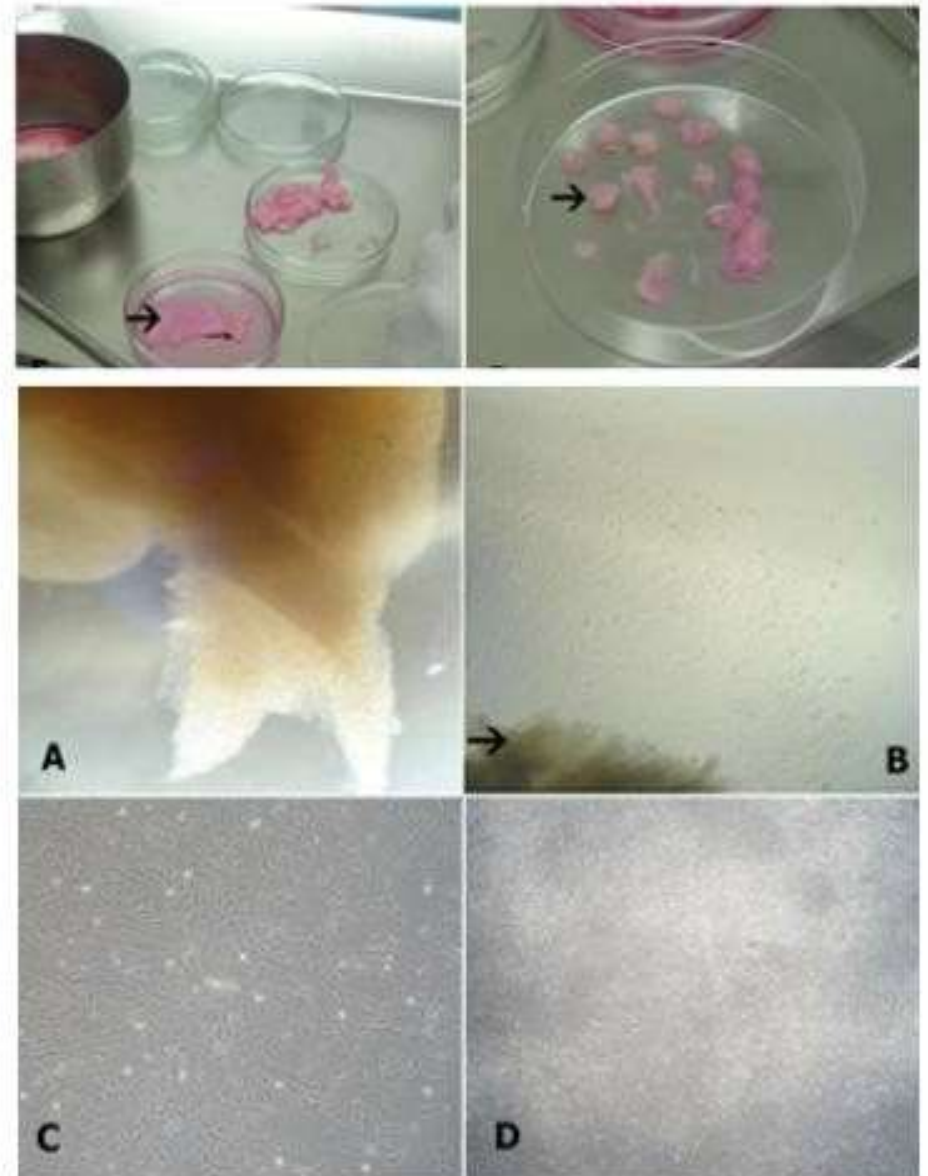
	Primary Cells	Cell Lines
Cell Division <i>In Vitro</i>	Limited	Indefinite
More Closely Mimic <i>In Vivo</i> Conditions	Yes	No
Chromosomal Aberrations	Minimal	Several
Provide High Quality Data on Interactive Pathways, Processes, and Function	Retain functional enzymatic and signaling pathways of parent tissue	Potentially provide misleading data due to aberrant enzymatic or signaling pathway activities
Authentication required before use	No, if bought commercially	Yes, mandated by many government institutes and scientific journals
Care and Maintenance of the Cells	Can be complex and difficult, especially in maintaining differentiated state	Easy to maintain or propagate

1- Primary Culture

- There are two basic methods for obtaining primary culture:

1. **Explant cultures:**

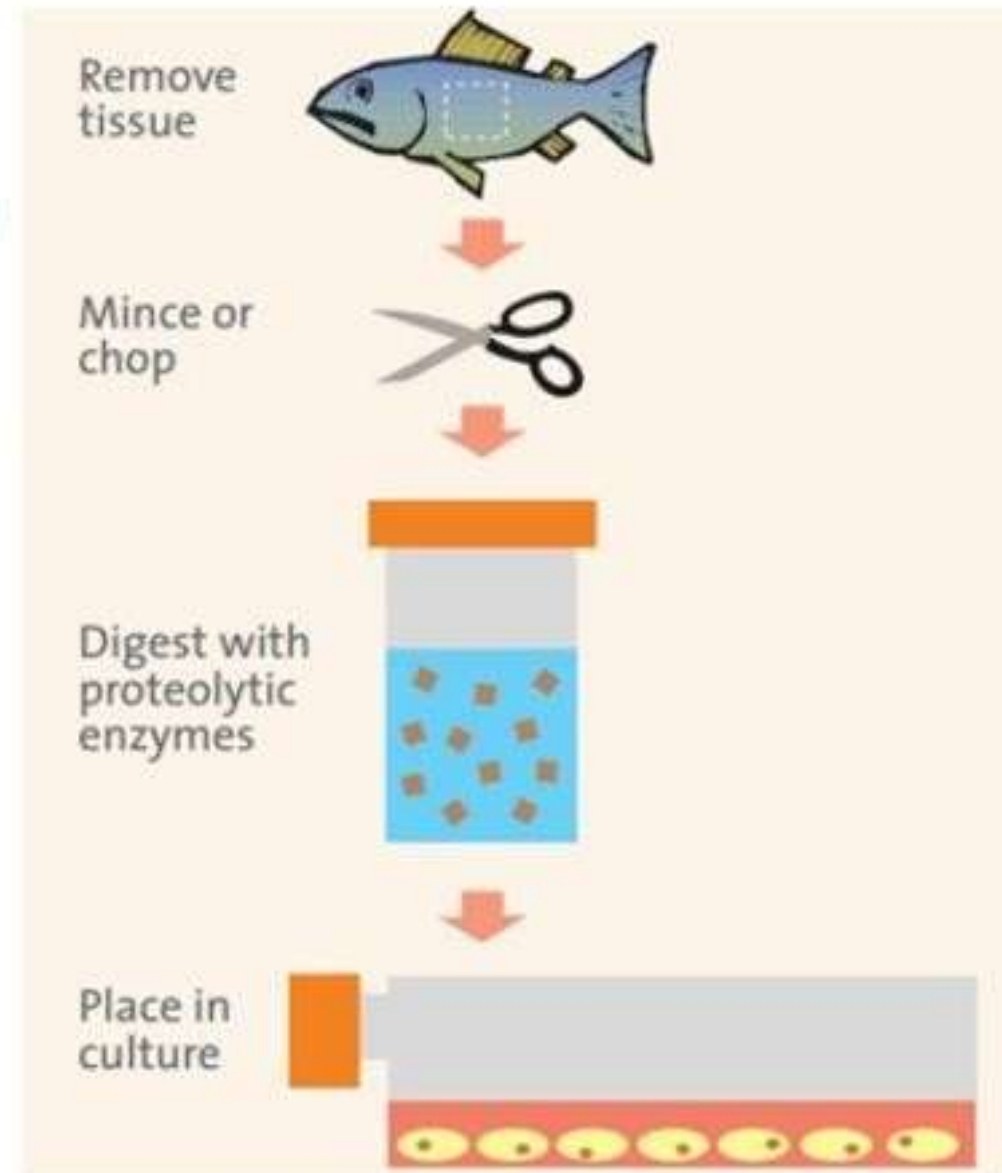
- Small pieces of tissue are attached (using plasma clots or fibrinogen) to a glass or treated plastic culture vessel and immersed in culture medium
- After a few days individual cells will move from the tissue explant out onto the culture vessel surface or substrate where they will begin to divide and grow



-1 Primary Culture

2. Enzymatic dissociation:

- More widely used
- speeds up the process by adding digesting (proteolytic) enzymes such as trypsin or collagenase to the tissue fragments to dissolve the cement holding the cells together
- This creates a suspension of single cells that are then placed into culture vessels containing culture medium and allowed to grow and divide



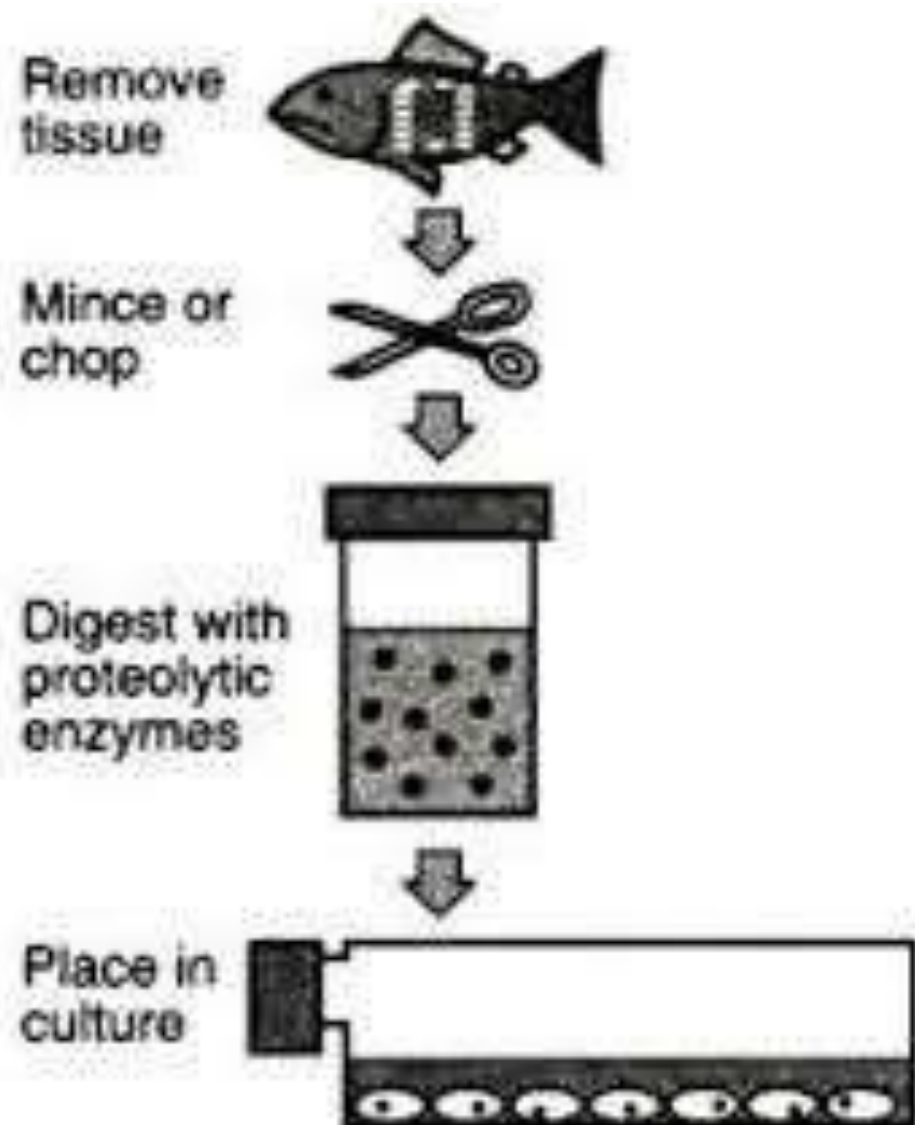
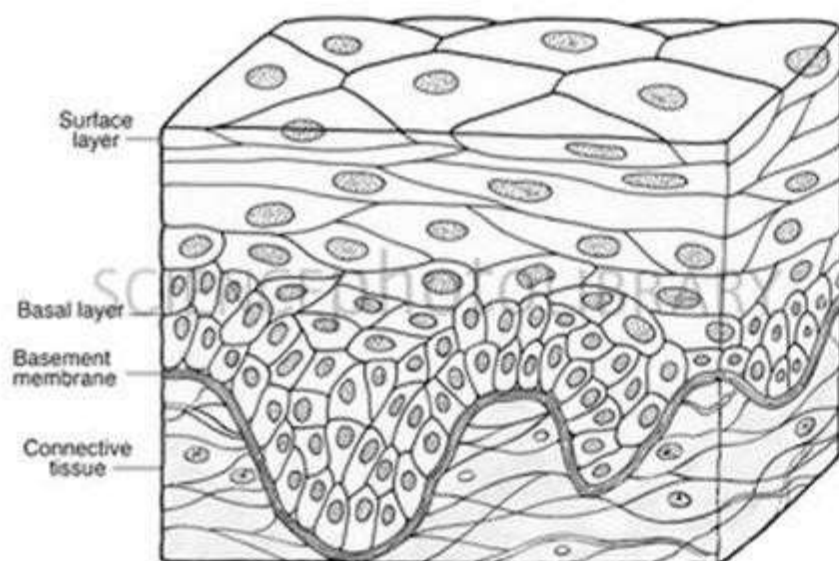


Fig. 10.8: Obtaining primary cultures by enzymatic dissociation



Tissue dissociation by enzymatic digestion

Aim: Dissociate cells-cell contacts, i.e. epithelium



Involve several steps including:

Collagenase treatment

to digest extracellular matrix and

Protease (e.g. trypsin)

to disperse tightly associated cells.

Result: Disruption of stable cell-cell interactions (i.e. gap junctions, tight junctions, adherens junctions) which play a crucial role for the biological activity or structural characteristics of the cells and tissues

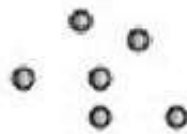
Considered: Substantial manipulation



**peripheral ganglia
with nerve fibers**

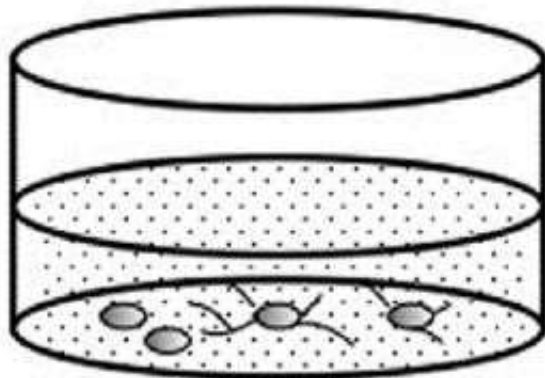


**collagenase /
trypsin**



**ganglion
neurons**

dissociated cell culture

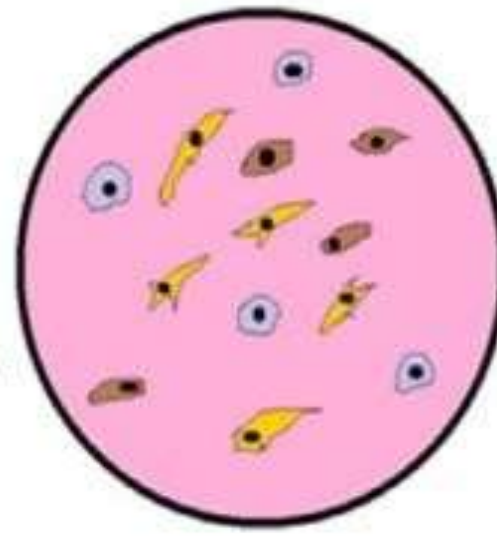


explant culture

(A)

Proteolysis leads to
production of
single cell suspension

Single cell
suspension



(B)

Culture surface

CKs and GFs

Explant tissue piece

Migrated cells

Migrated MSCs

Released CKs and GFs



Mesenchymal stem cells (MSCs)

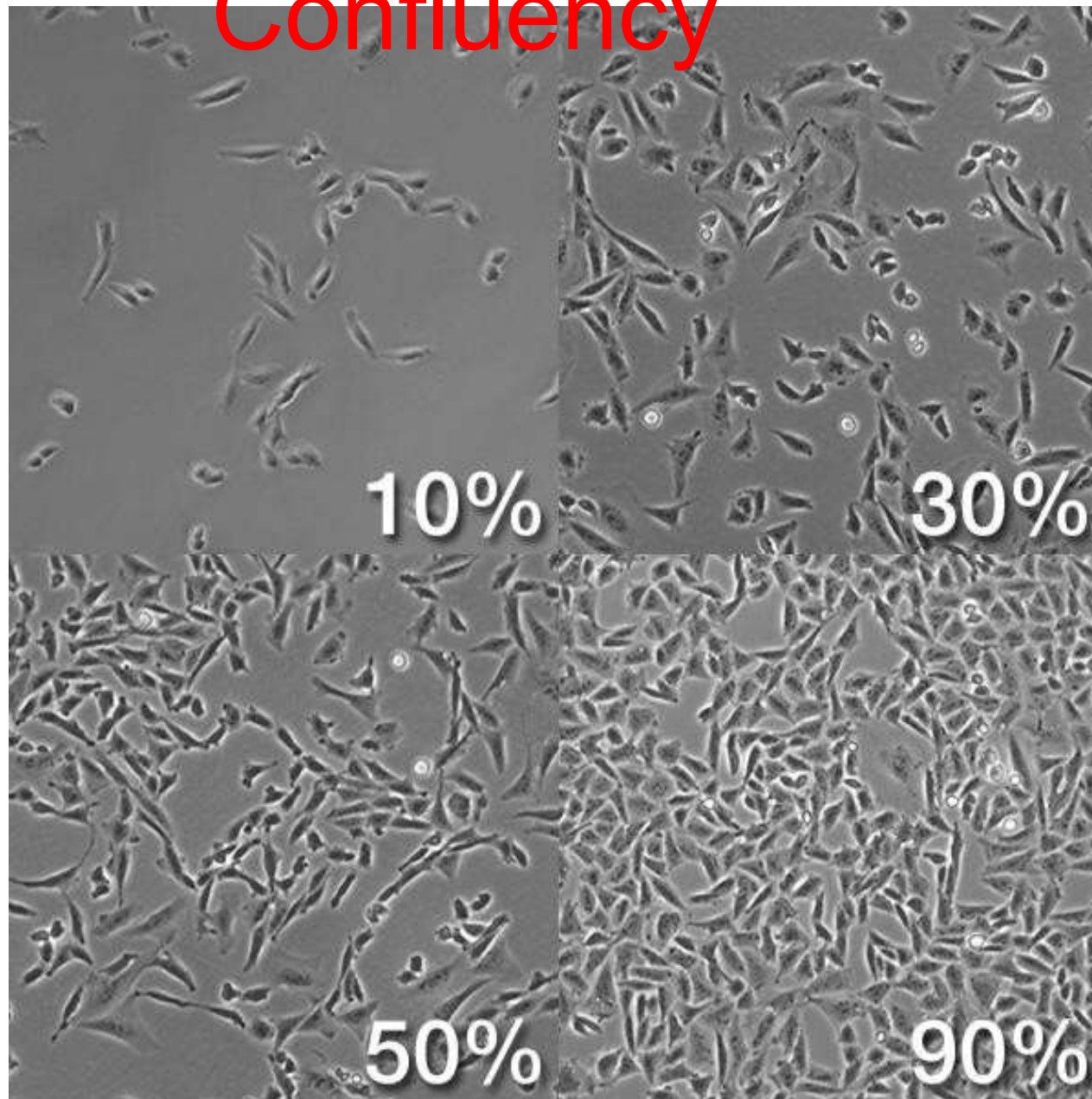


Other cells in the tissue

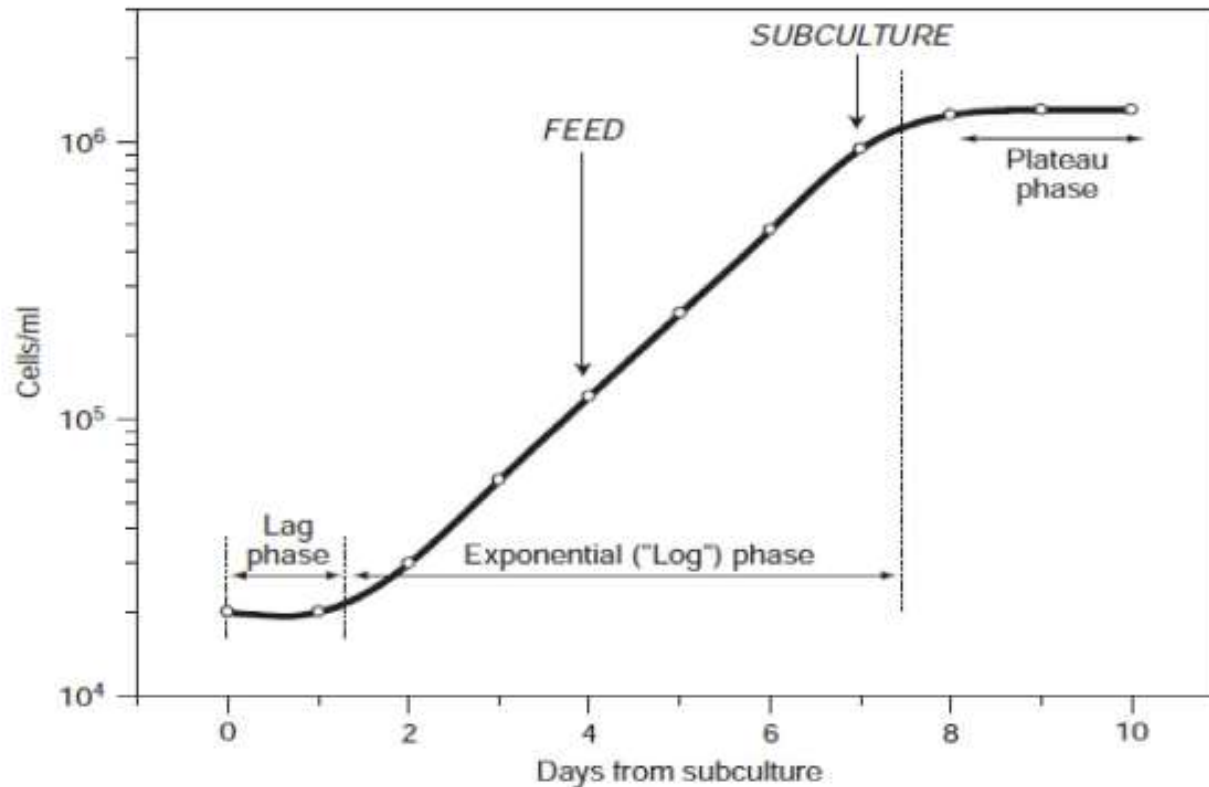


Cytokines (CKs) and Growth factors (GFs)

Cell Confluency



Growth curve and maintenance



Semilog plot of cell concentration versus time from subculture the lag phase, exponential phase, and plateau, and indicating times at which subculture and feeding should be performed.

Criteria for subculture

- **Density of culture**: Normal cell should be subcultured as soon as they reach confluence.
- **Exhaustion of medium**: It indicates that the medium require replacement. Usually a drop in PH is indicate that need of subculture.
- **Time since last subculture**. Routine subculture is best performed according to a strict schedule, so that reproducible behaviour is achieved and monitored.

Why grow animal cells in culture?

- to investigate the normal physiology or biochemistry of cells. For example, metabolic pathways can be investigated by radioactively labeled substrates applied and subsequently looking at products
- to test the effects of compounds on specific cell types. Such compounds may be metabolites, hormones or growth factors. Similarly, potentially toxic or mutagenic compounds may be evaluated in cell culture

Why grow animal cells in culture?

- to produce artificial tissue by combining specific cell types in sequence. This has considerable potential for production of artificial skin for use in treatment of burns.
- to synthesize valuable products (biologicals) from large-scale cell cultures. The biologicals encompass a broad range of cell products and include specific proteins or viruses that require animal cells for propagation.

