Biyoteknolojik Uygulamalarda In vitro Deneysel Araştırma Teknikleri

2. Ders

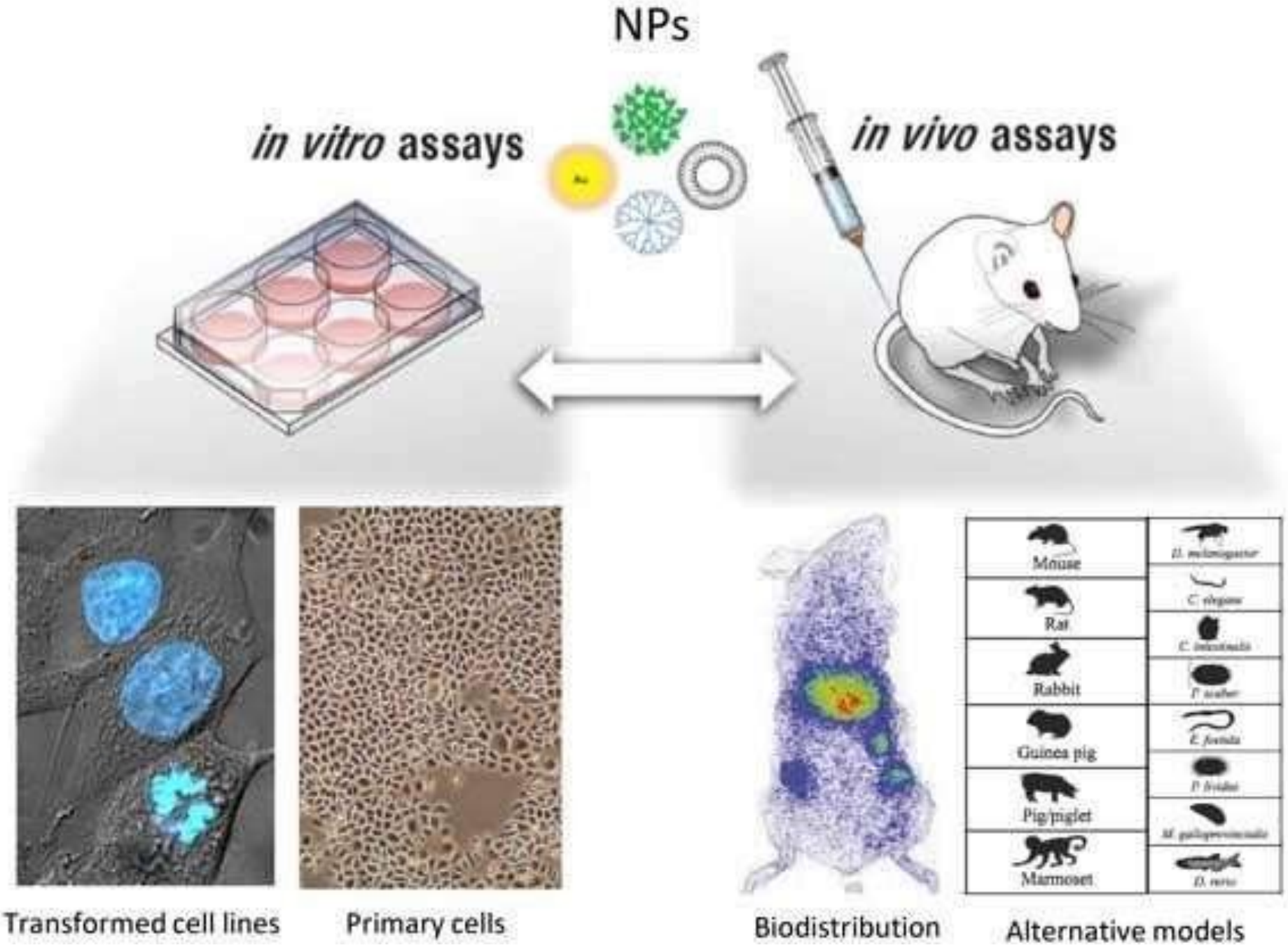
Cell Culture

- 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').


NPs
in vitro assays
in vivo assays
Mouse
Rat
Rabbit
Guinea pig
Pig/piglet
Marmoset
Transformed cell lines
Primary cells
Biodistribution
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 there 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).

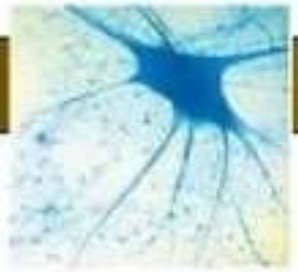
History of cell culture

1885
Roux
Cells can
live outside
the body

1907
Harrison
Nerve
outgrowth

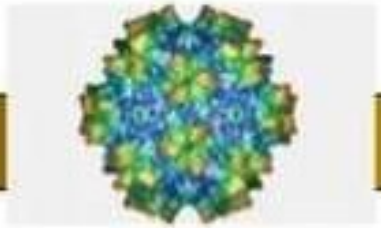
1952
Gey
Established
HeLa cells

1955
Eagle
Defined
medium



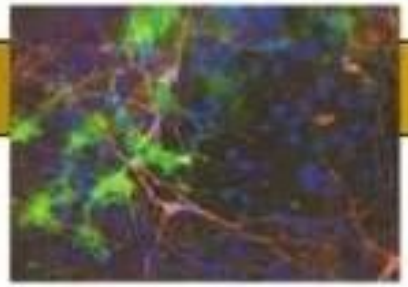
Late '50s and '60s
Stoker, Dulbecco,
Green
Viral transformation

1961
Hayflick/Moorhead
Finite number
of divisions



1964
Littlefield
Somatic cell
hydrids

1965
Ham
Clonal
growth of
mammalian cells



1976
Sato
Hormones
And growth
Factors required in
SF medium

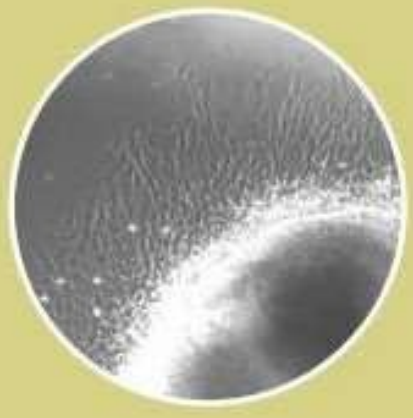
1977
Wigler
And Axel
Transfections

1986
Martin/Evans
Mouse ES cells

1998
Thomson
And Gearhart
Human ES cells

Establishment of Tissue Culture

Organ Culture



Primary Explant

Cell Culture

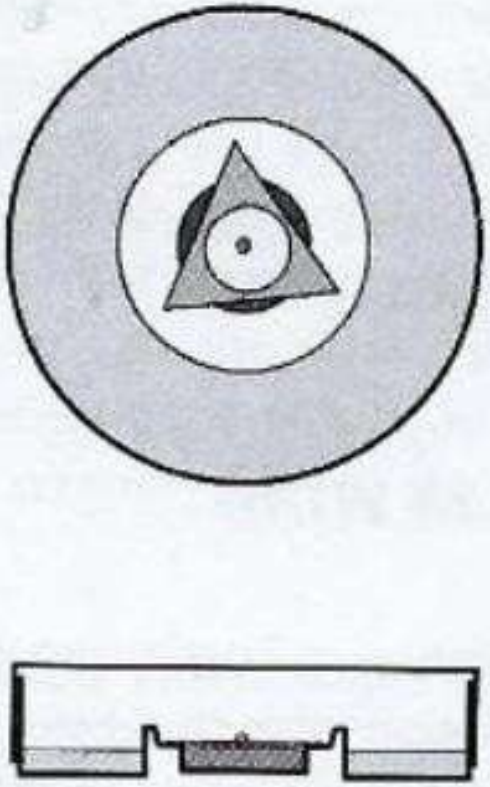
Tissue Culture:

- 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.

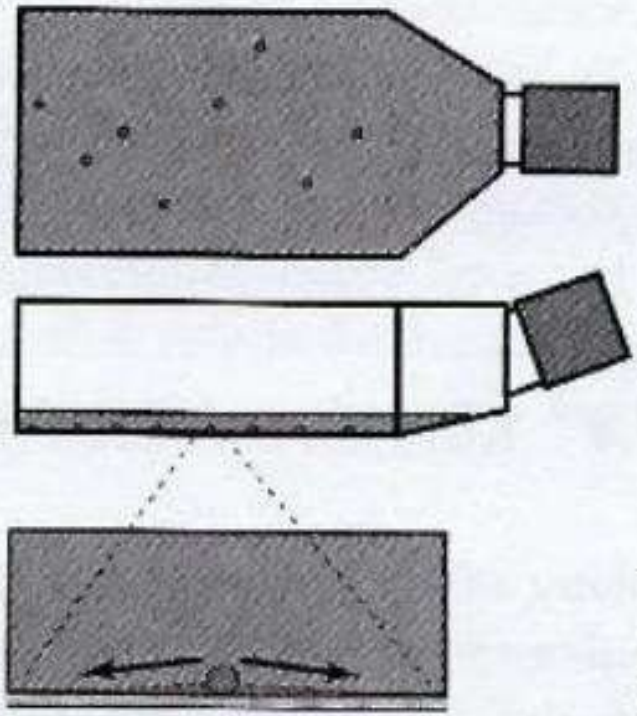
- Cell culture: explant/outgrowth from the primary explant is dispersed (mechanically/enzymatically) into a cell suspension that can be cultured as an adherent monolayer.

ORGAN CULTURE



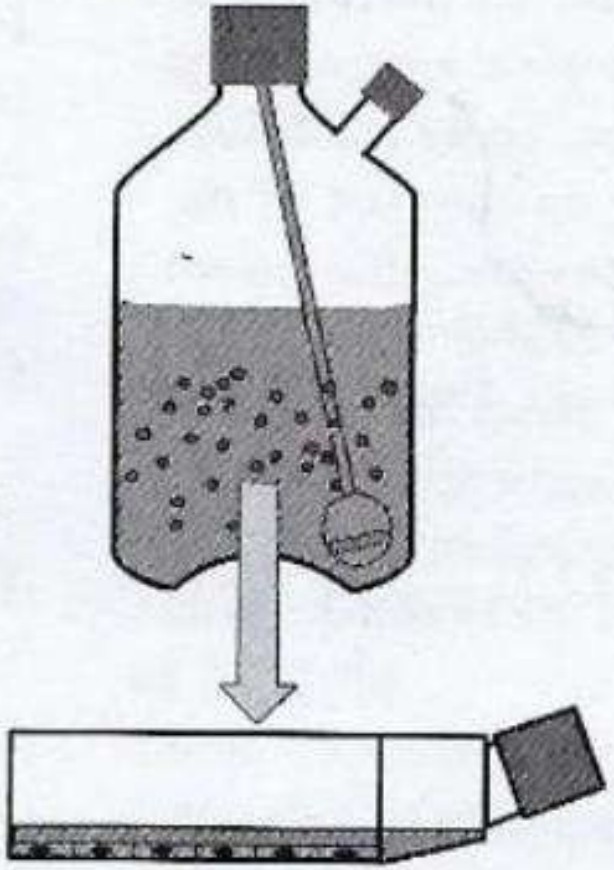
Tissue at gas-liquid interface; histological structure maintained

EXPLANT CULTURE



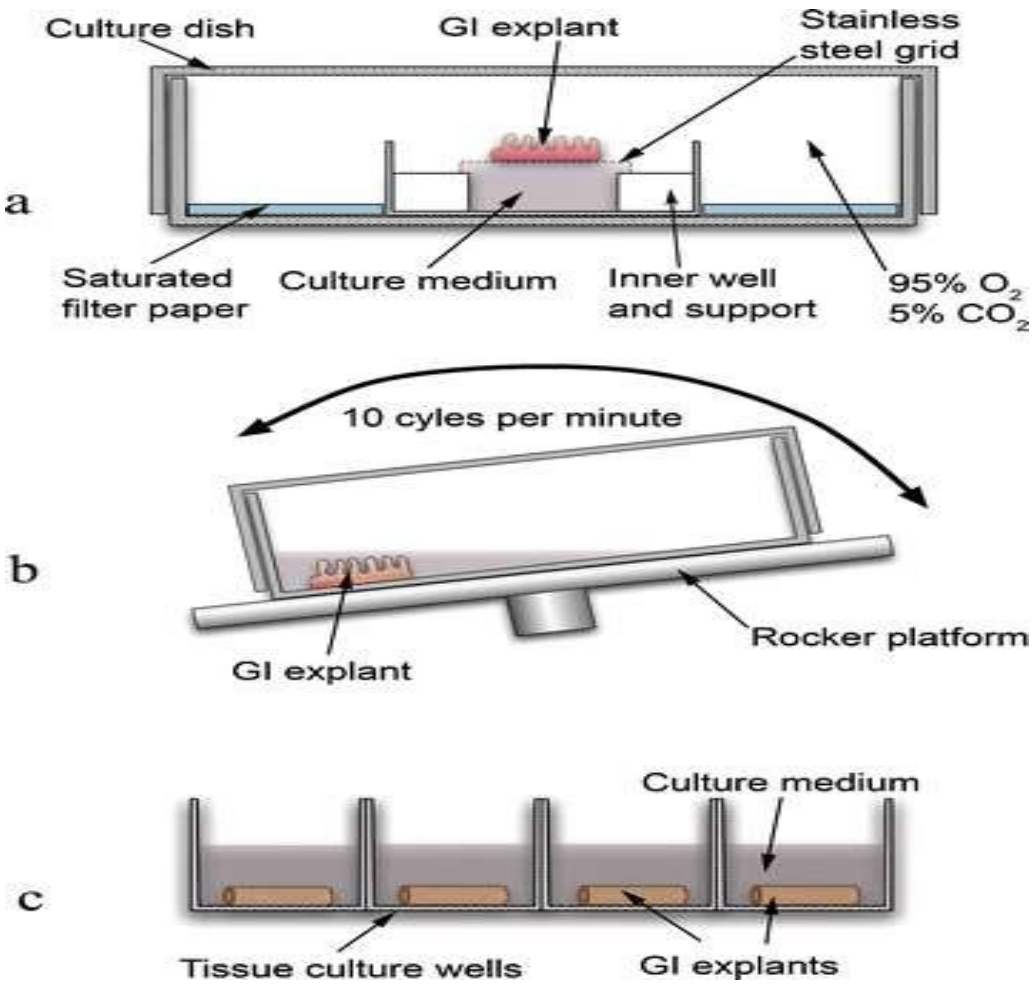
Tissue at solid-liquid interface; cells migrate to form outgrowth

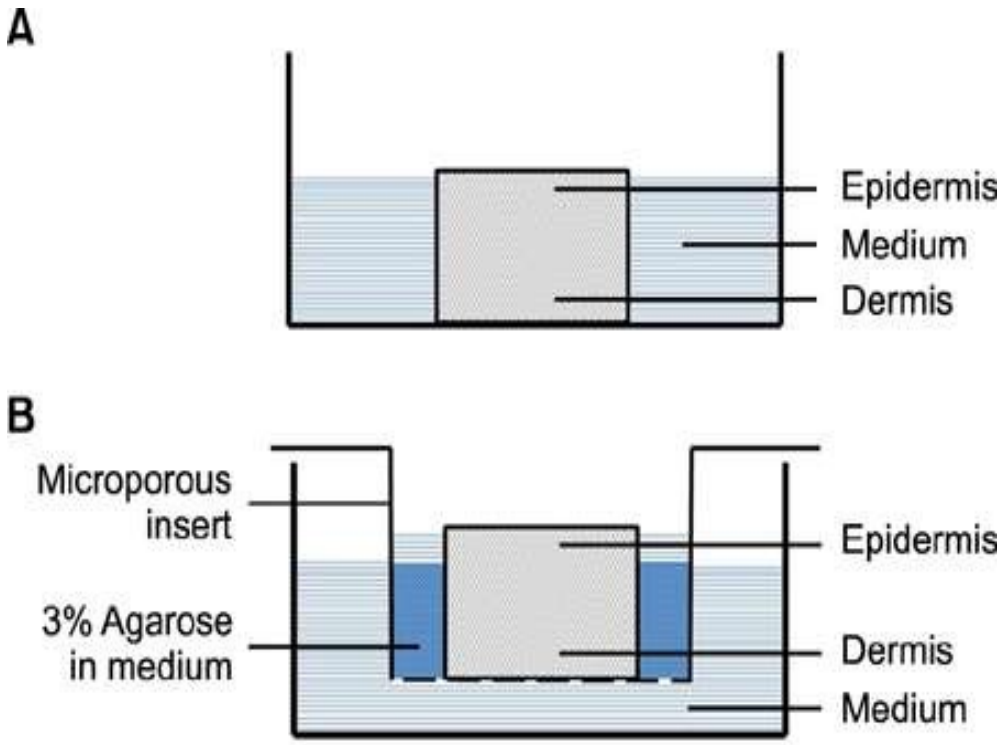
DISSOCIATED CELL CULTURE



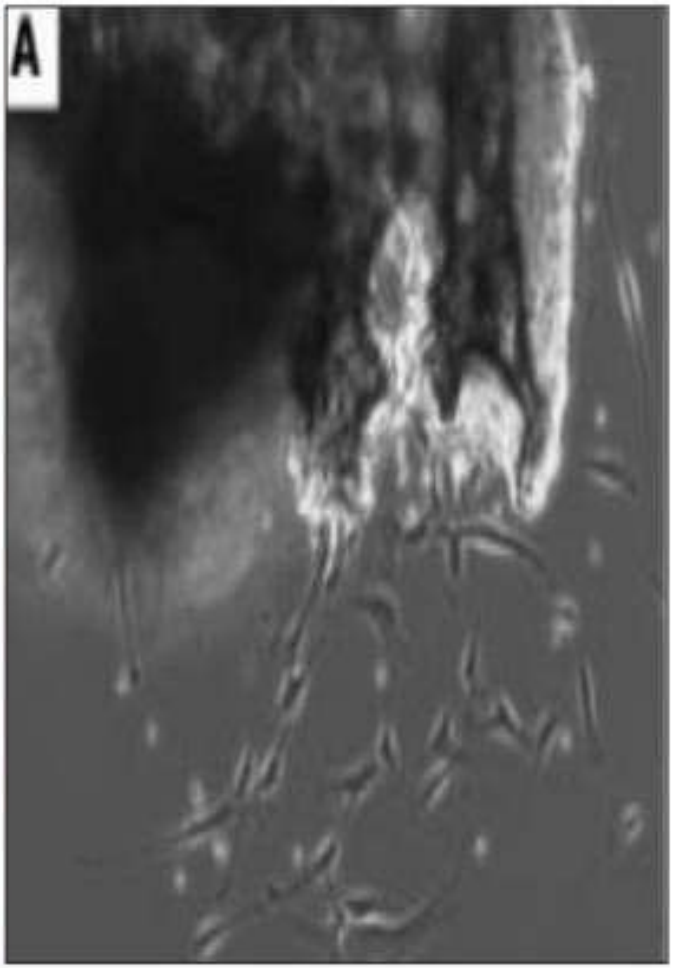
Disaggregated tissue; cells form monolayer at solid-liquid interface

Organ Culture

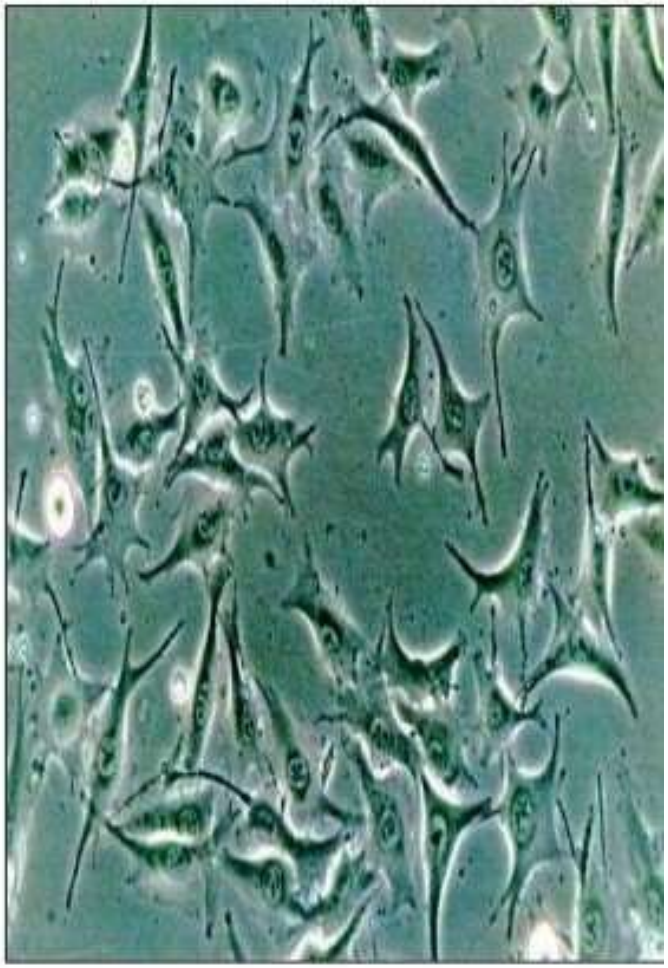




Human skin in medium



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:

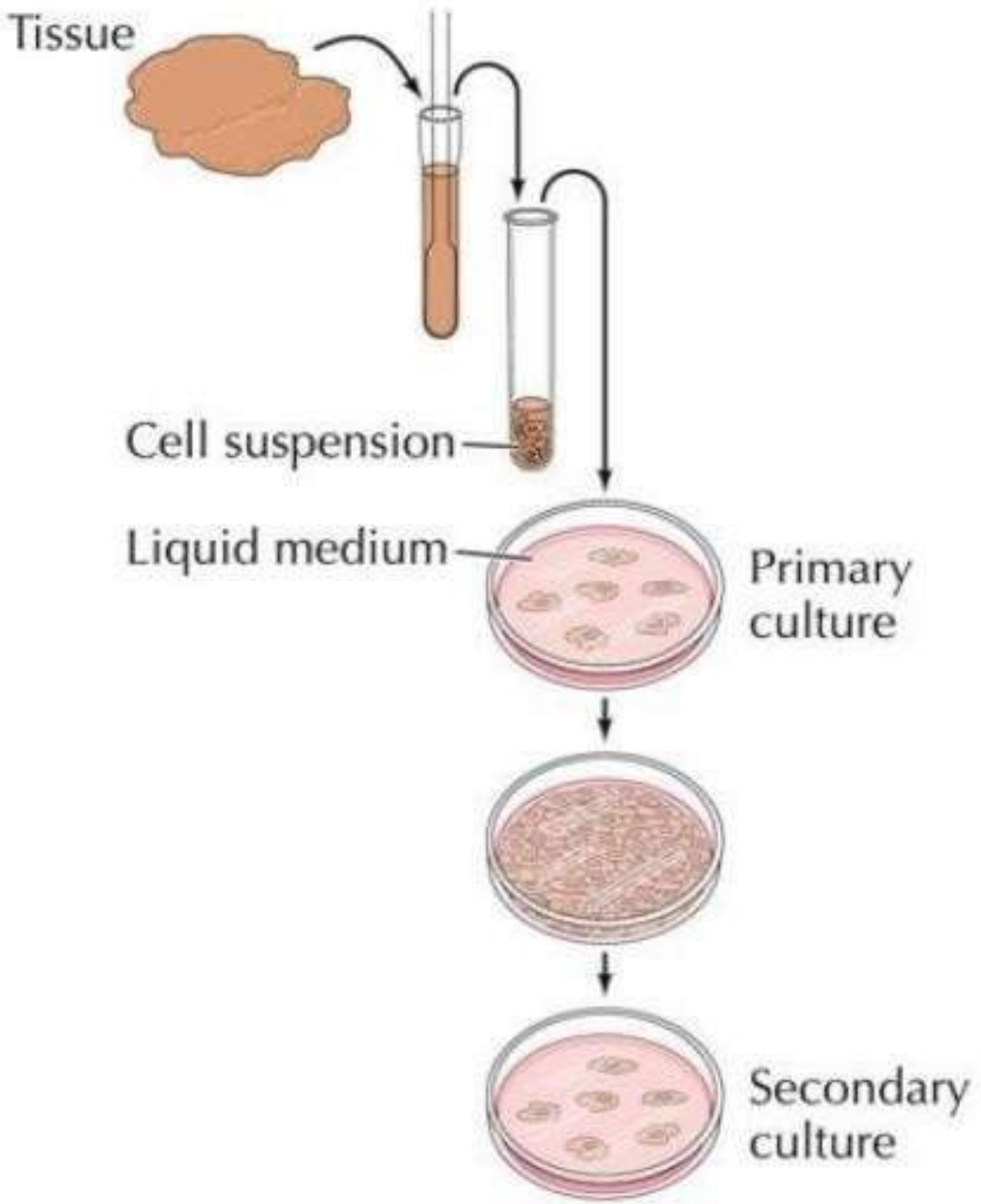
1. Primary cells
2. Cell lines
3. Continouos cell lines

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 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.



THE CELL, Fourth Edition, Figure 1.41 © 2006 ASM Press and Sinauer Associates, Inc.

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

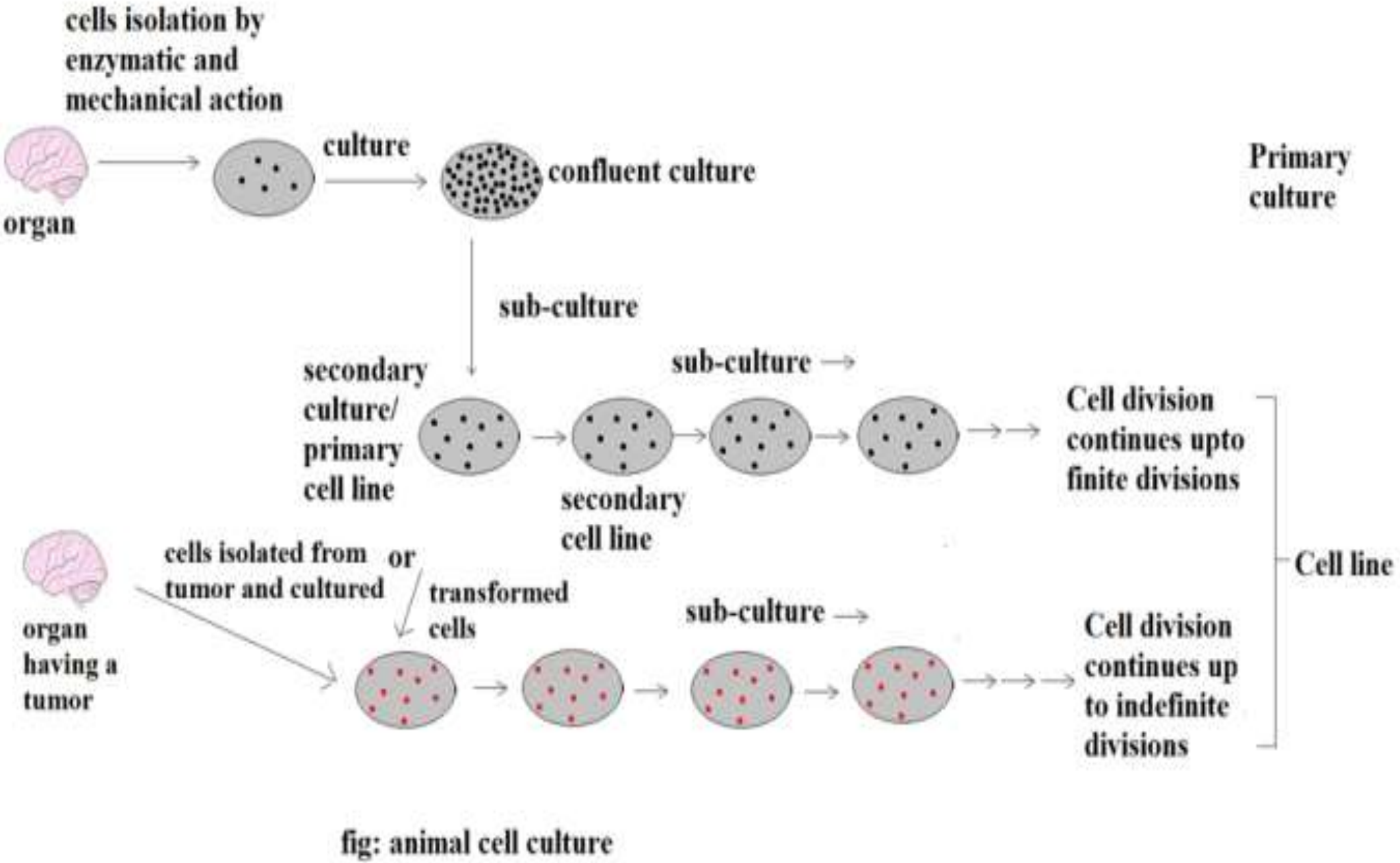


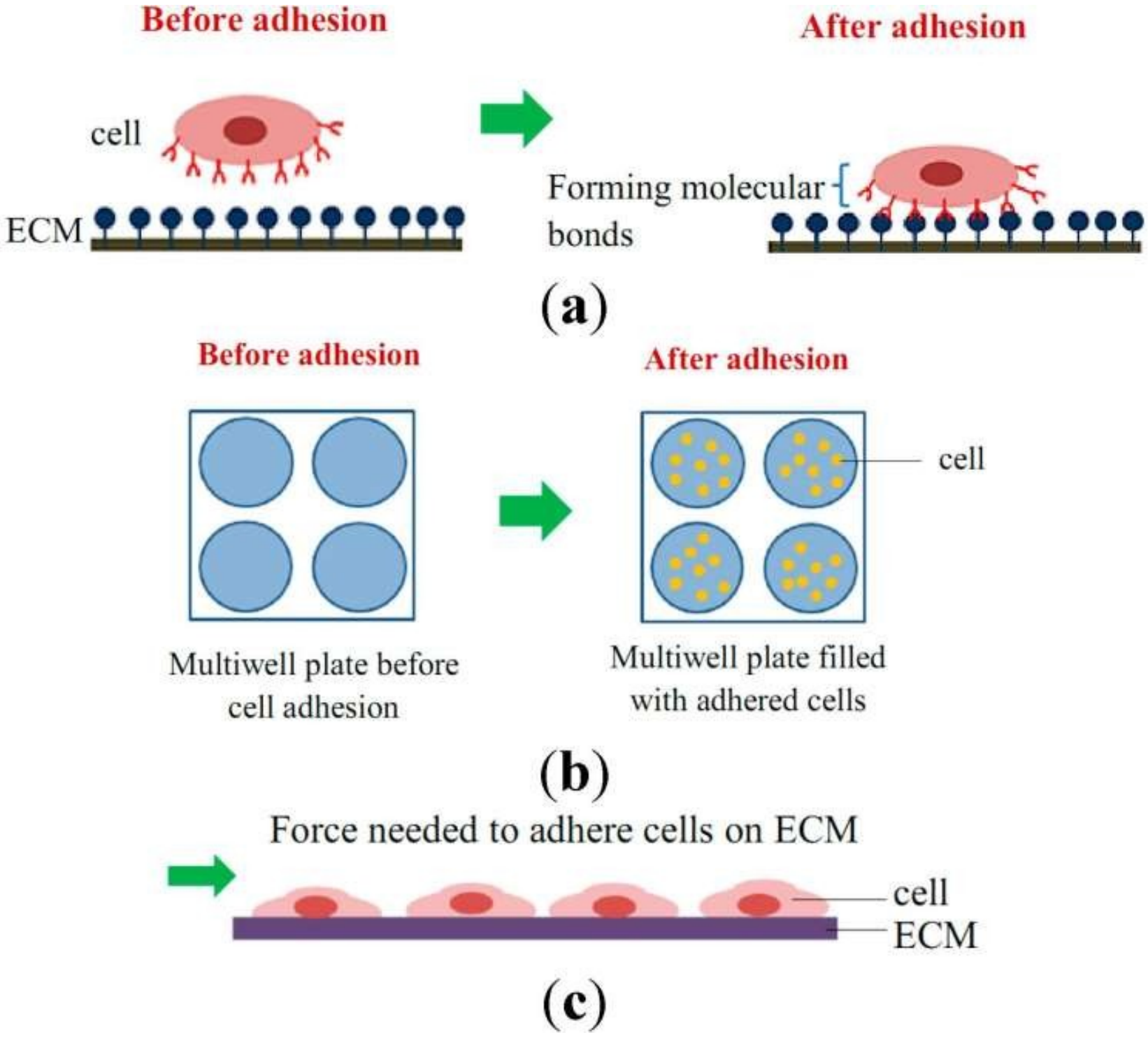
fig: animal cell culture

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

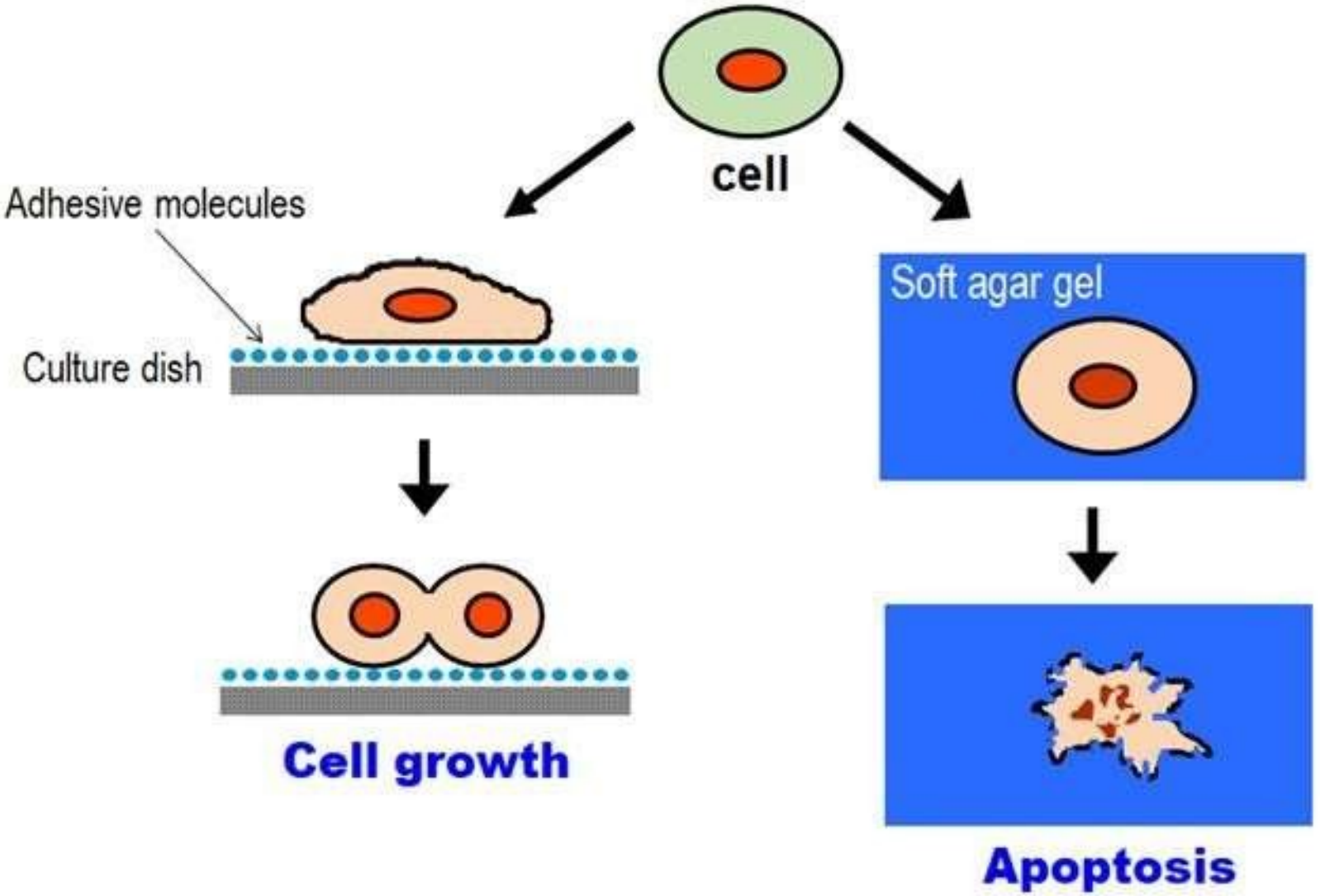
1. Adherent monolayer cells
2. Suspension cells

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.


Before adhesion
After adhesion
cell
ECM
Forming molecular bonds
(a)
Before adhesion
After adhesion
cell
Multiwell plate before cell adhesion
Multiwell plate filled with adhered cells
(b)
Force needed to adhere cells on ECM
cell
ECM
(c)

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



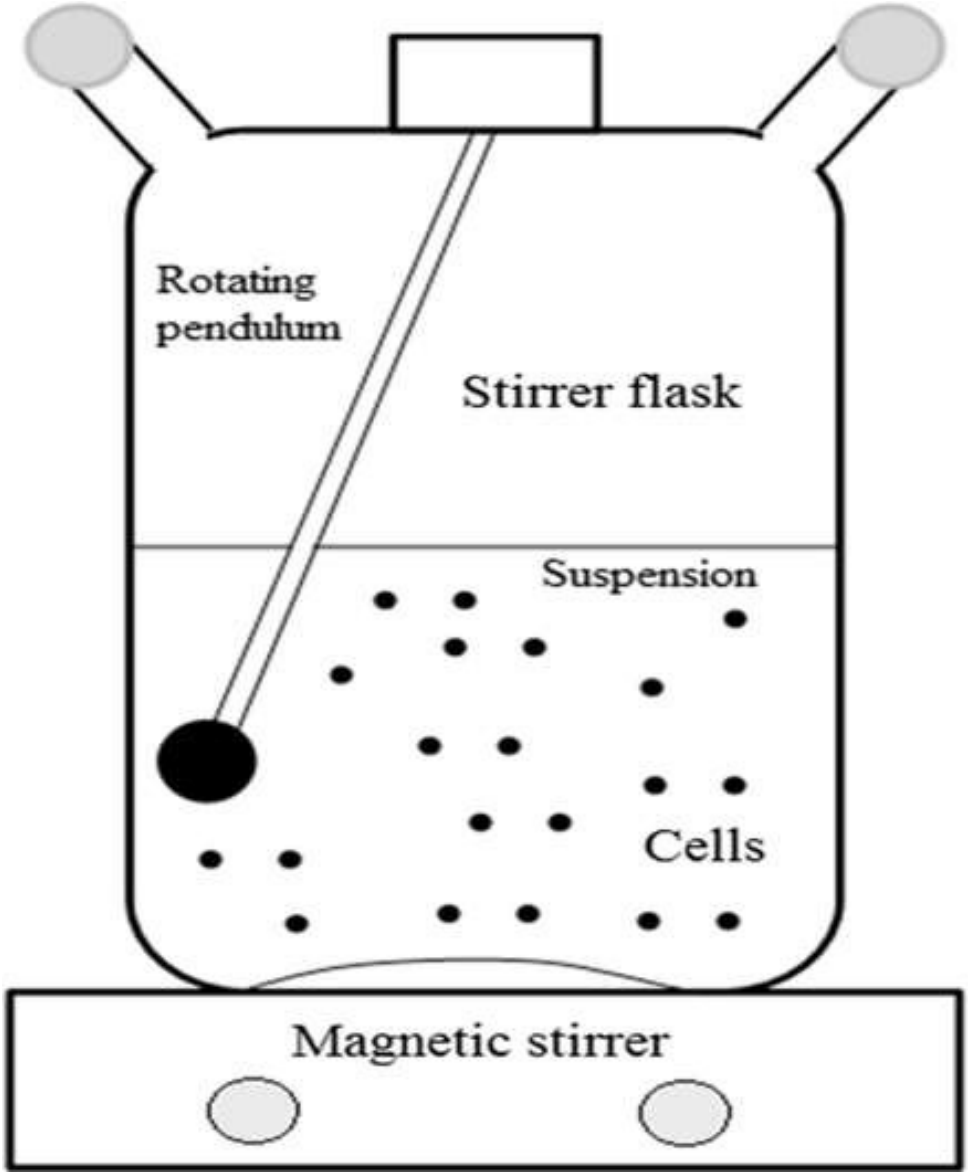
Suspension cells

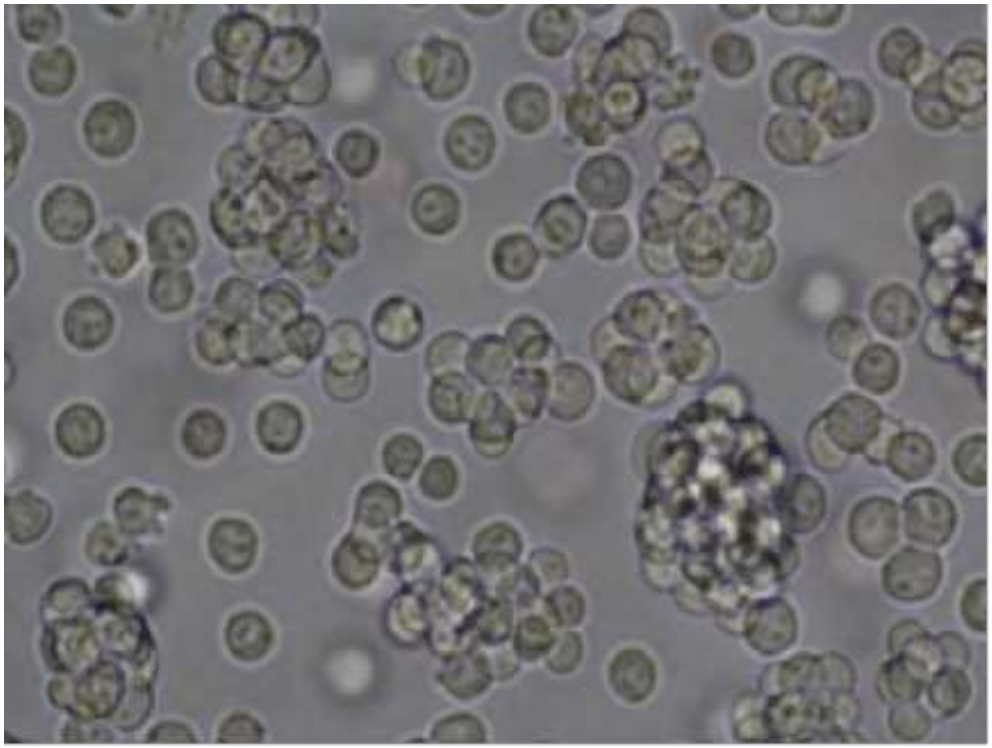
- Suspension cells do not attach to the surface of the culture vessels.
- These cells are also called anchorage independent or non- adherent cells which can be grown floating in the culture medium.
- Hematopoietic stem cells (derived from blood, spleen and bone marrow) and tumor cells can be grown in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

Suspension Cells

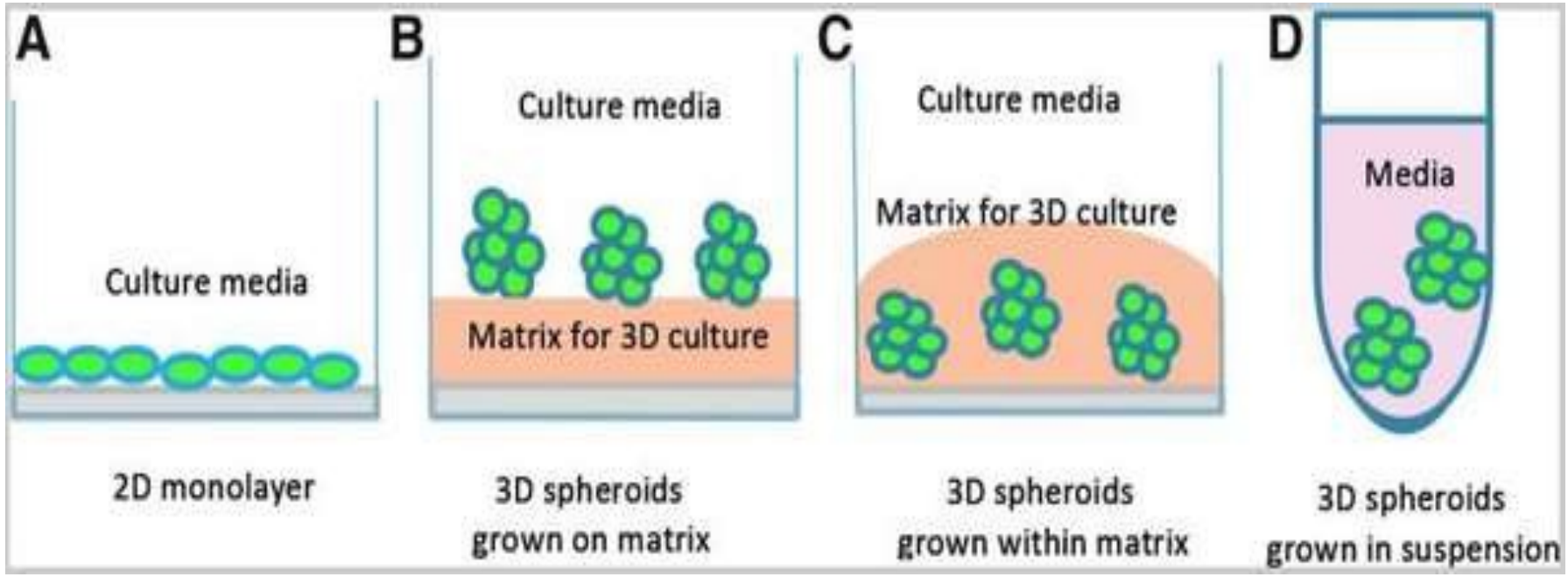
- These cells grow much faster which do not require the frequent replacement of the medium and can be easily maintained.
- These are of homogeneous types and enzyme treatment is not required for the dissociation of cells; similarly these cultures have short lag period.

Suspension Cells

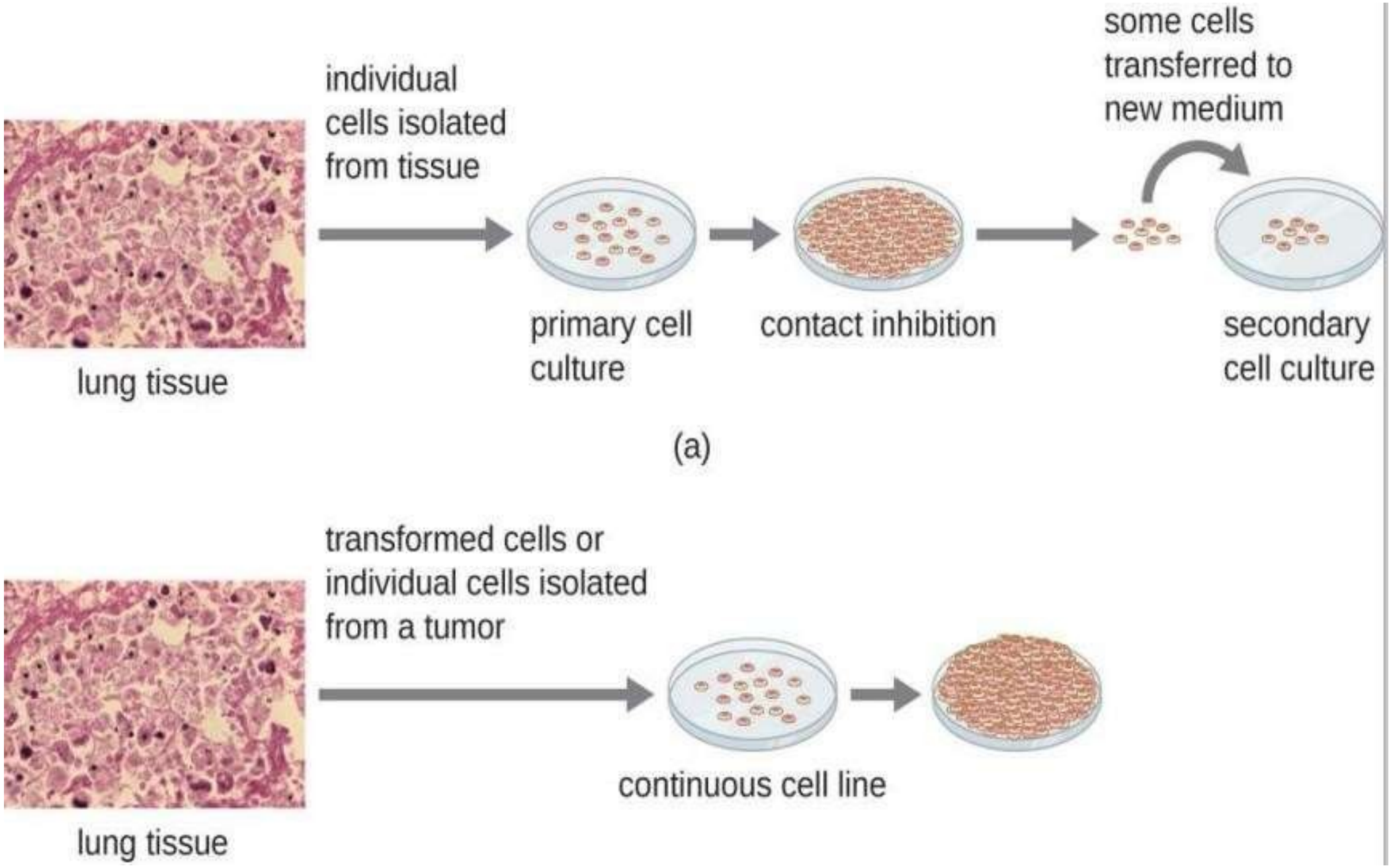




Monolayer Cells vs Suspension Cells



Contact Inhibition



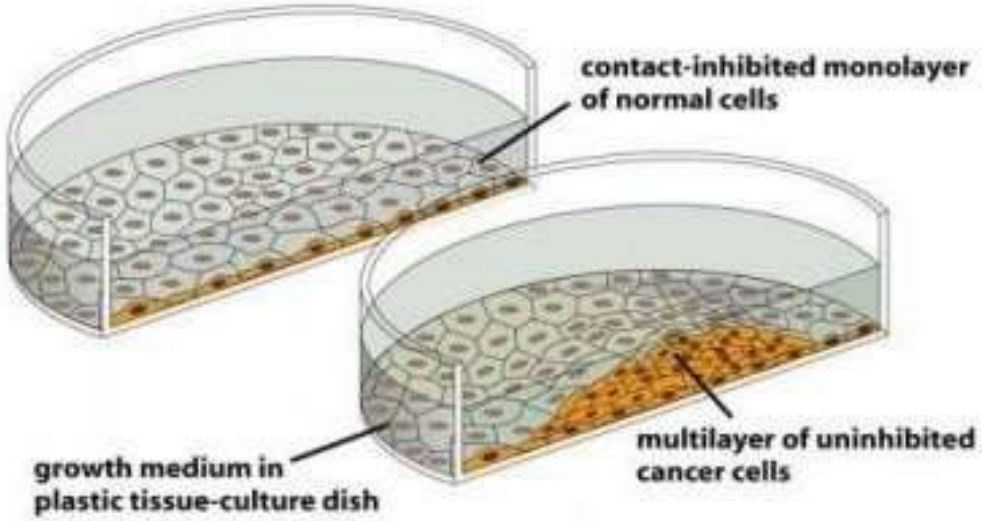
Demonstrating Contact Inhibition

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**

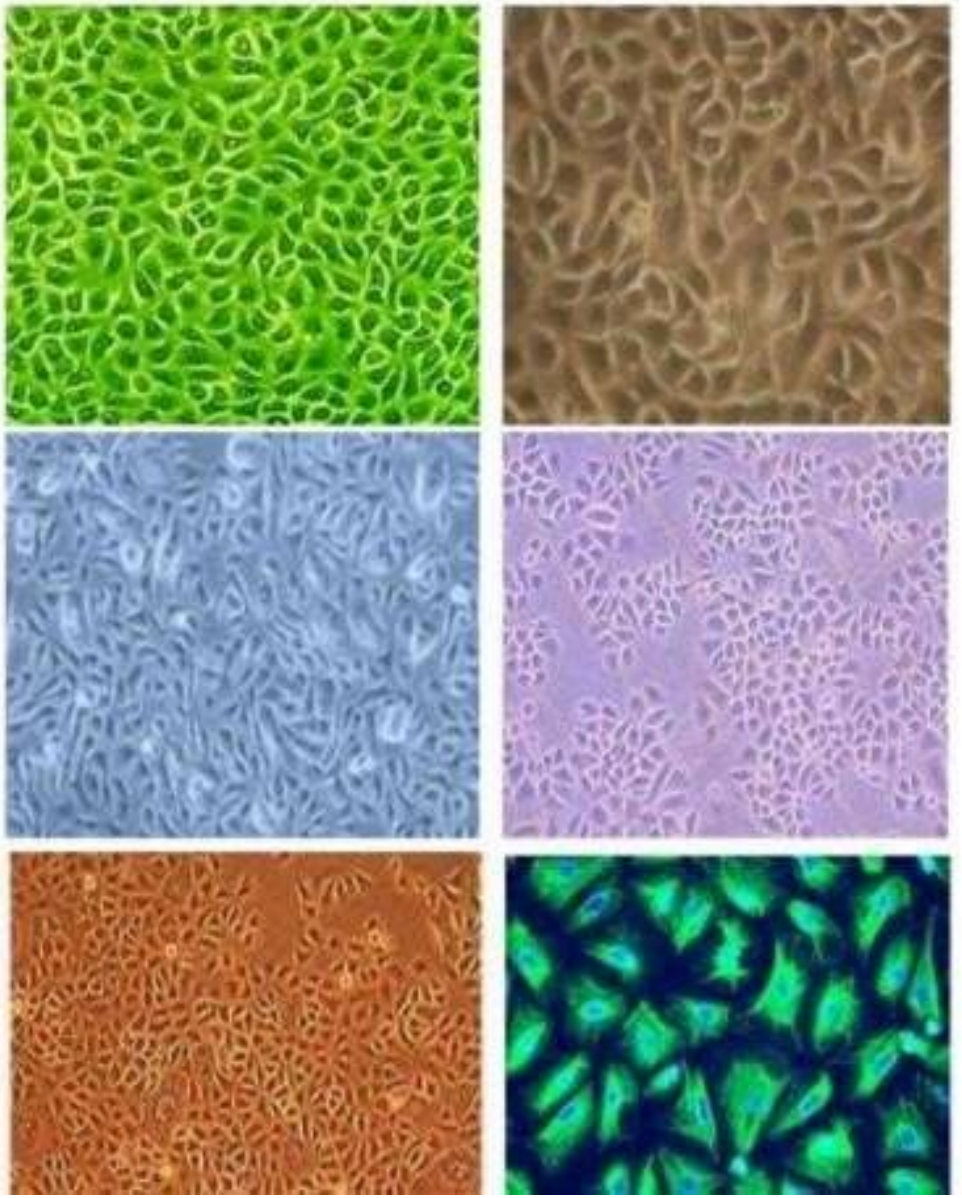
- **Fibroblast-like**
- **Epithelial-like**
- **Lymphoblast-like**

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 celllines 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 celllines in many ways

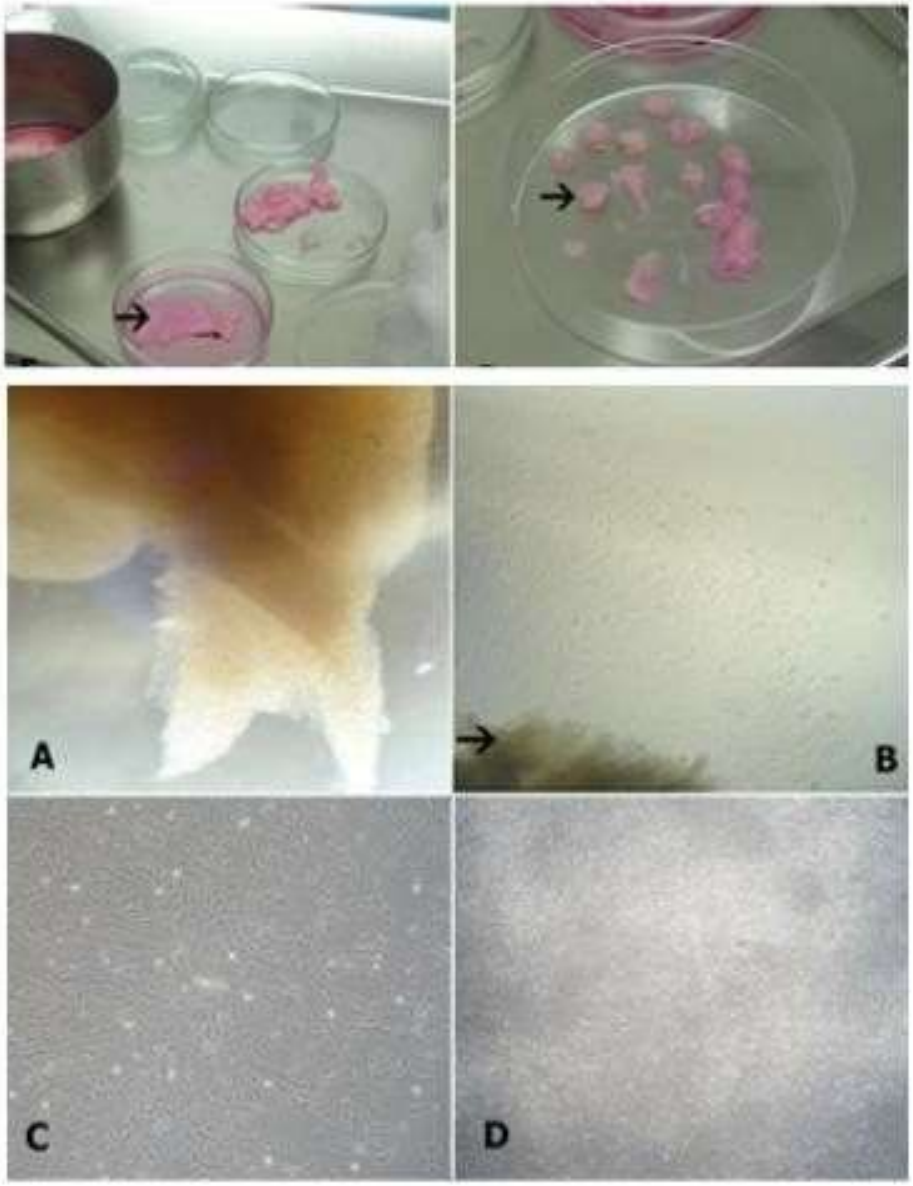
- Primary cells retain the natural feature of the tissue where they had been isolated. And due to this character, they are physiologically normal and thus theoretically provide unaltered, “natural” experimental results.
- Immortalized cell lines, on the other hand, are often transformed with other inducible 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 *In Vitro*	Limited	Indefinite
More Closely Mimic *In Vivo* 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

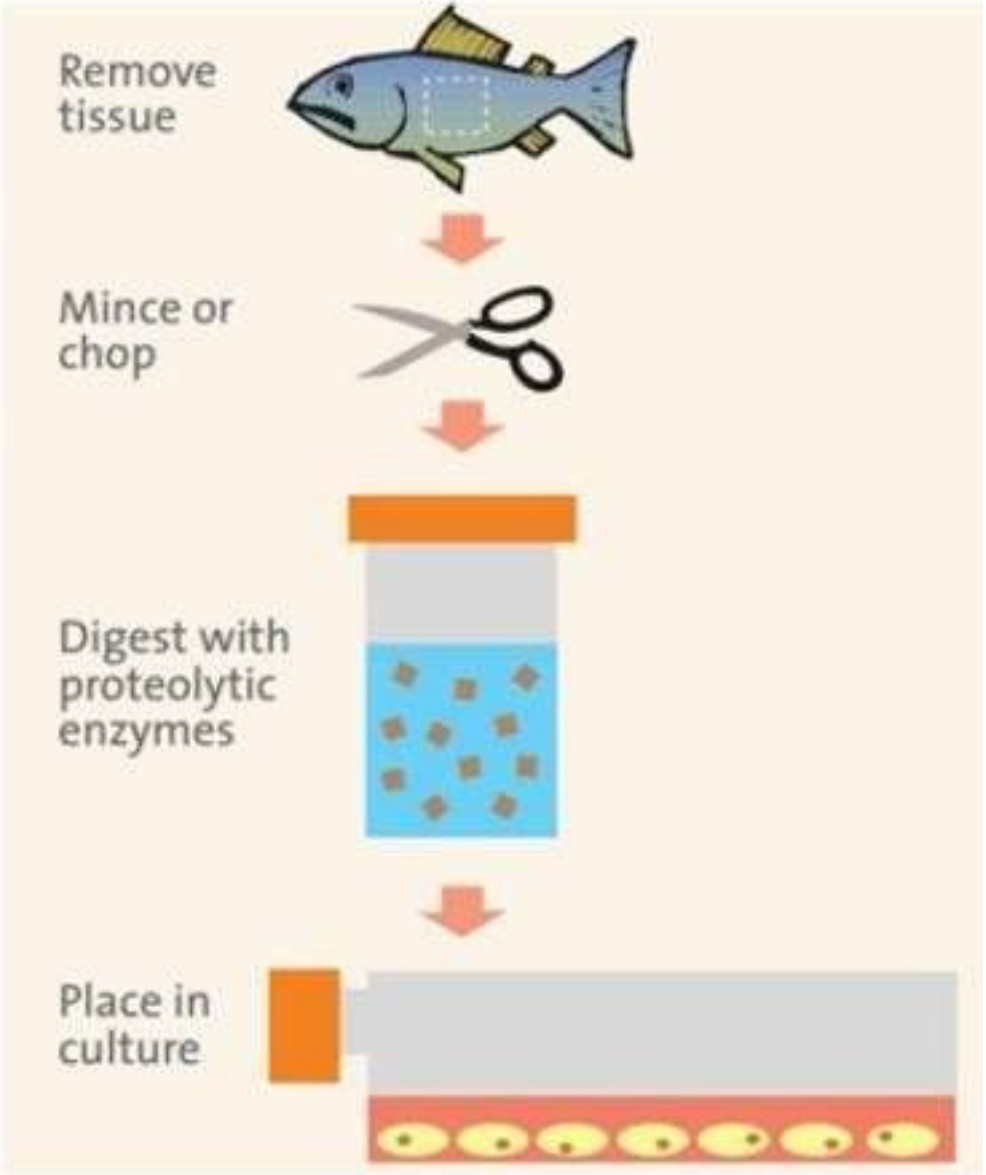
1- Primary Culture

- 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



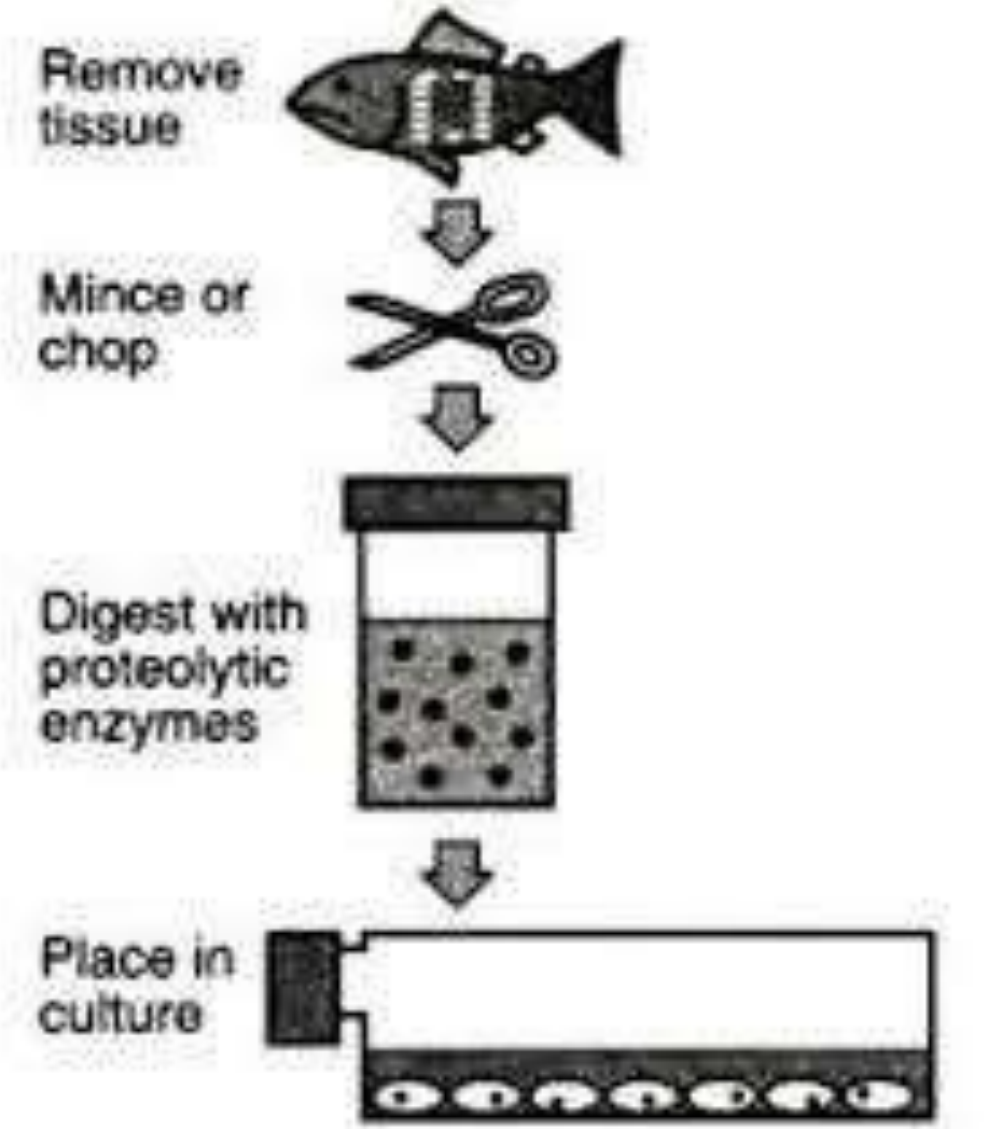
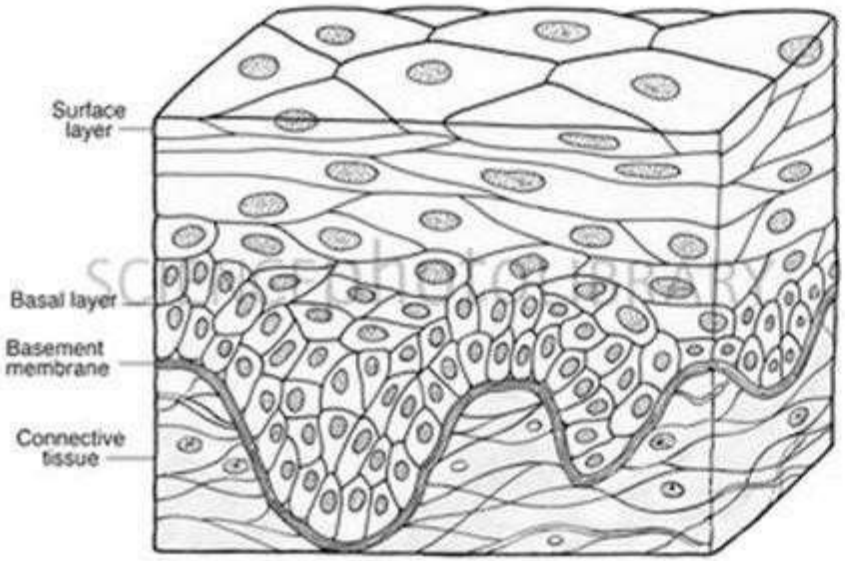


Fig. 10.8: Obtaining primary cultures by enzymatic dissociation

Tissue dissociation by enzymatic digestion

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



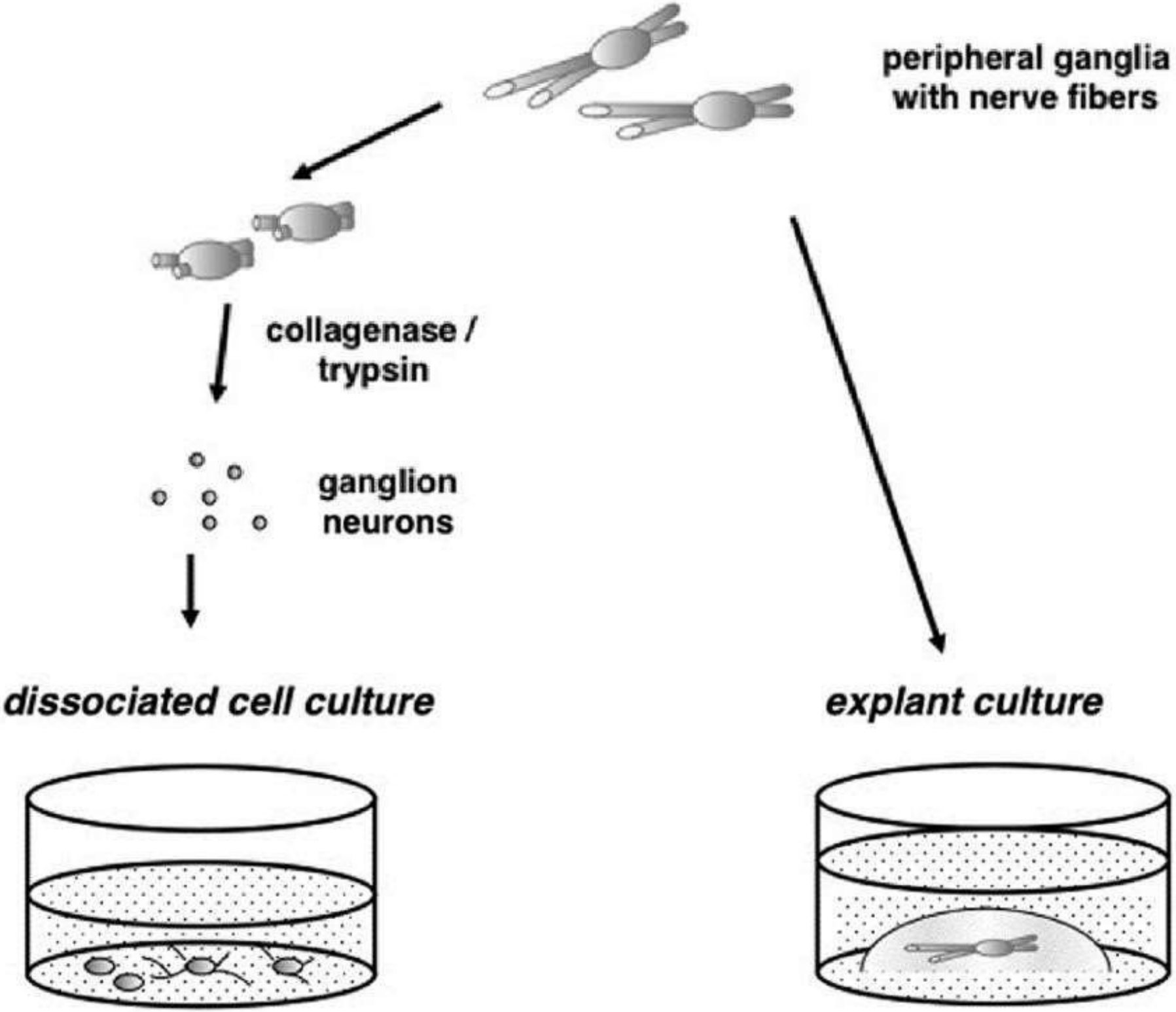
Involve several steps including:

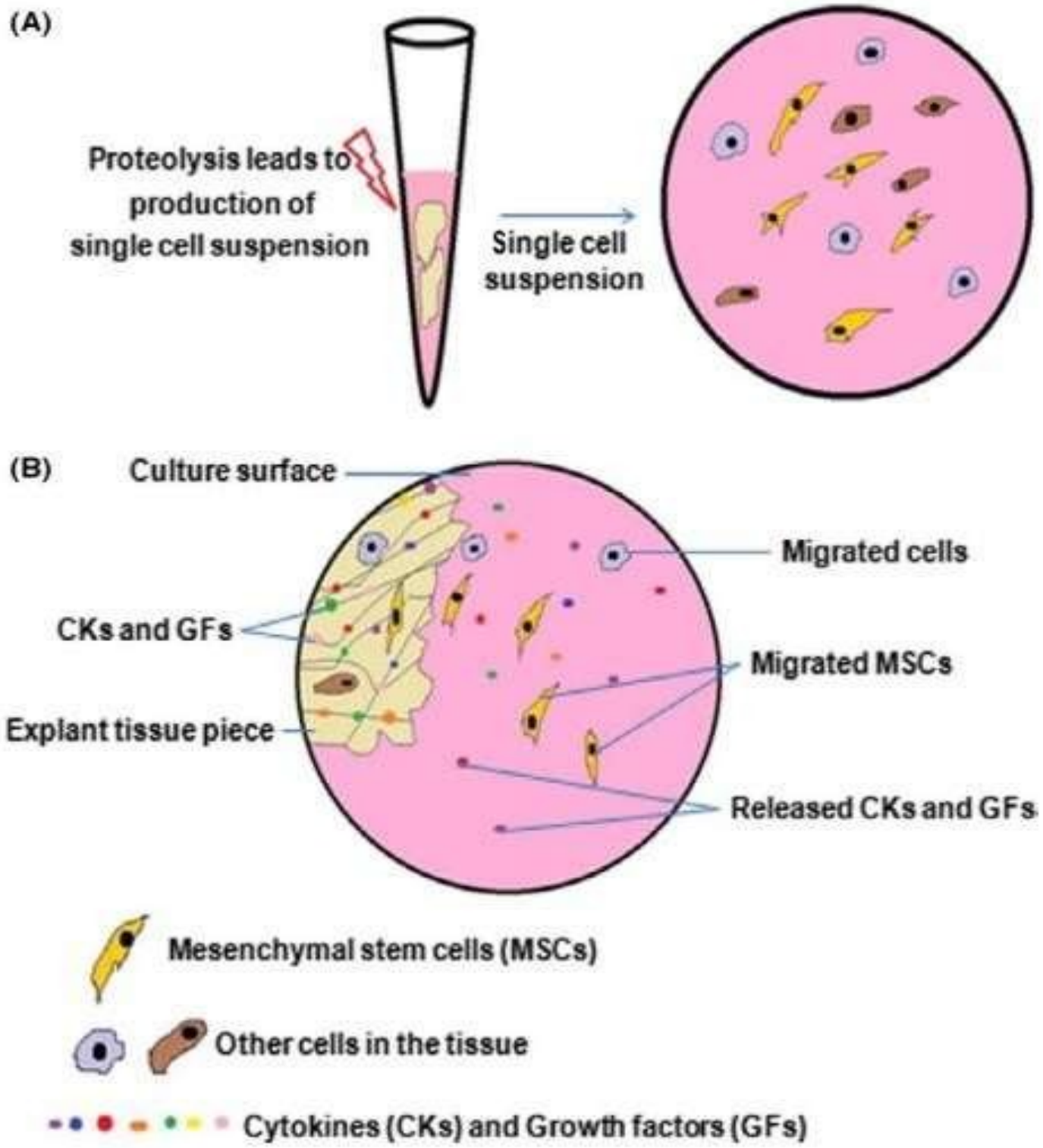
Collagenase treatment
to digest extracellular matrix and

Protease (e.g. trypsin)
to disperse tightly associated cells.

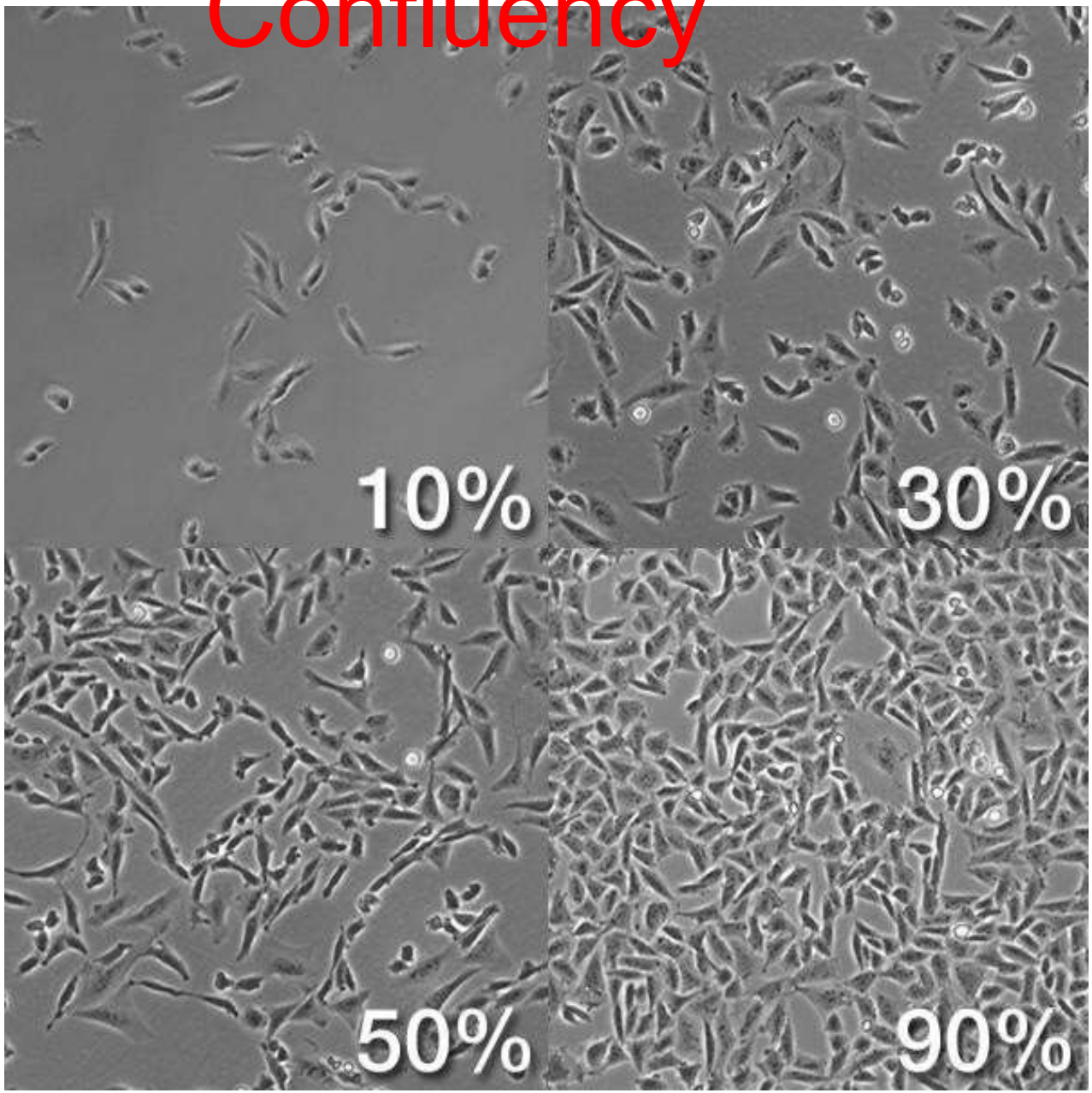
Result: Disruption of stable cell-cell interactions (i.e. gap junctions, tight junctions, adherens junctions) which play a crucial role for the biological activity or structural characteristics of the cells and tissues

Considered: Substantial manipulation

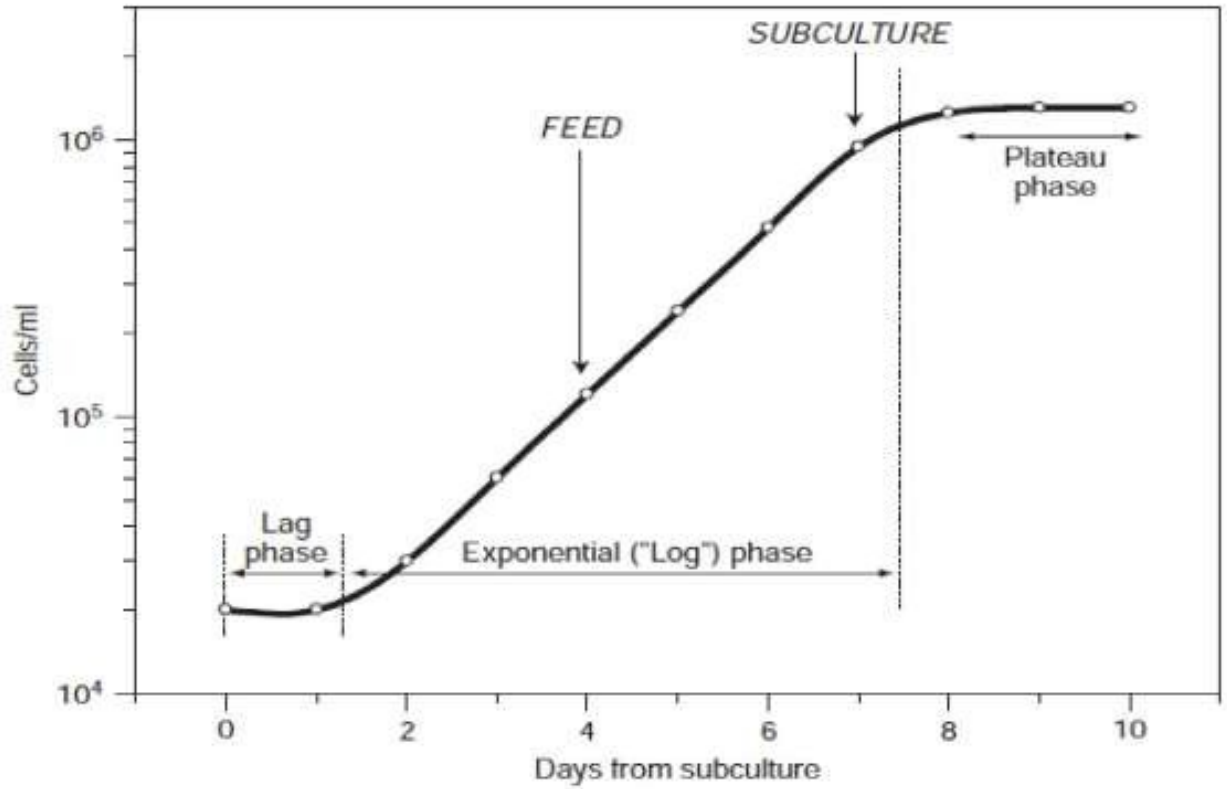

peripheral ganglia
with nerve fibers
collagenase /
trypsin
ganglion
neurons
dissociated cell culture
explant culture


(A)
Proteolysis leads to production of single cell suspension
Single cell suspension
(B)
Culture surface
Migrated cells
CKs and GFs
Migrated MSCs
Explant tissue piece
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.

Ref : R. Ian Freshney (2010). 6th edition

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.

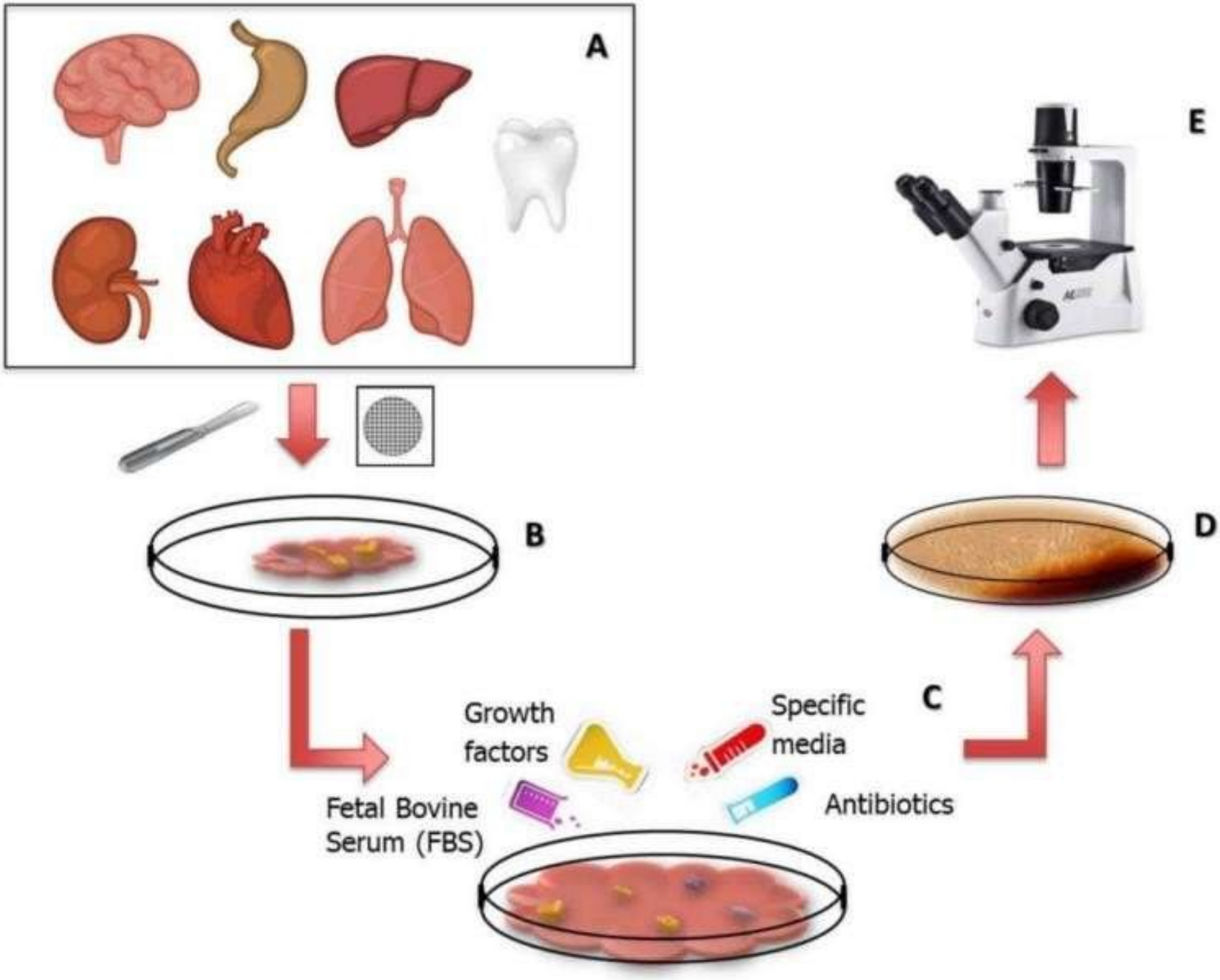
- **Time since last subculture**. Routine subculture is best performed according to a strict schedule, so that reproducible behaviour is achieved and monitored.

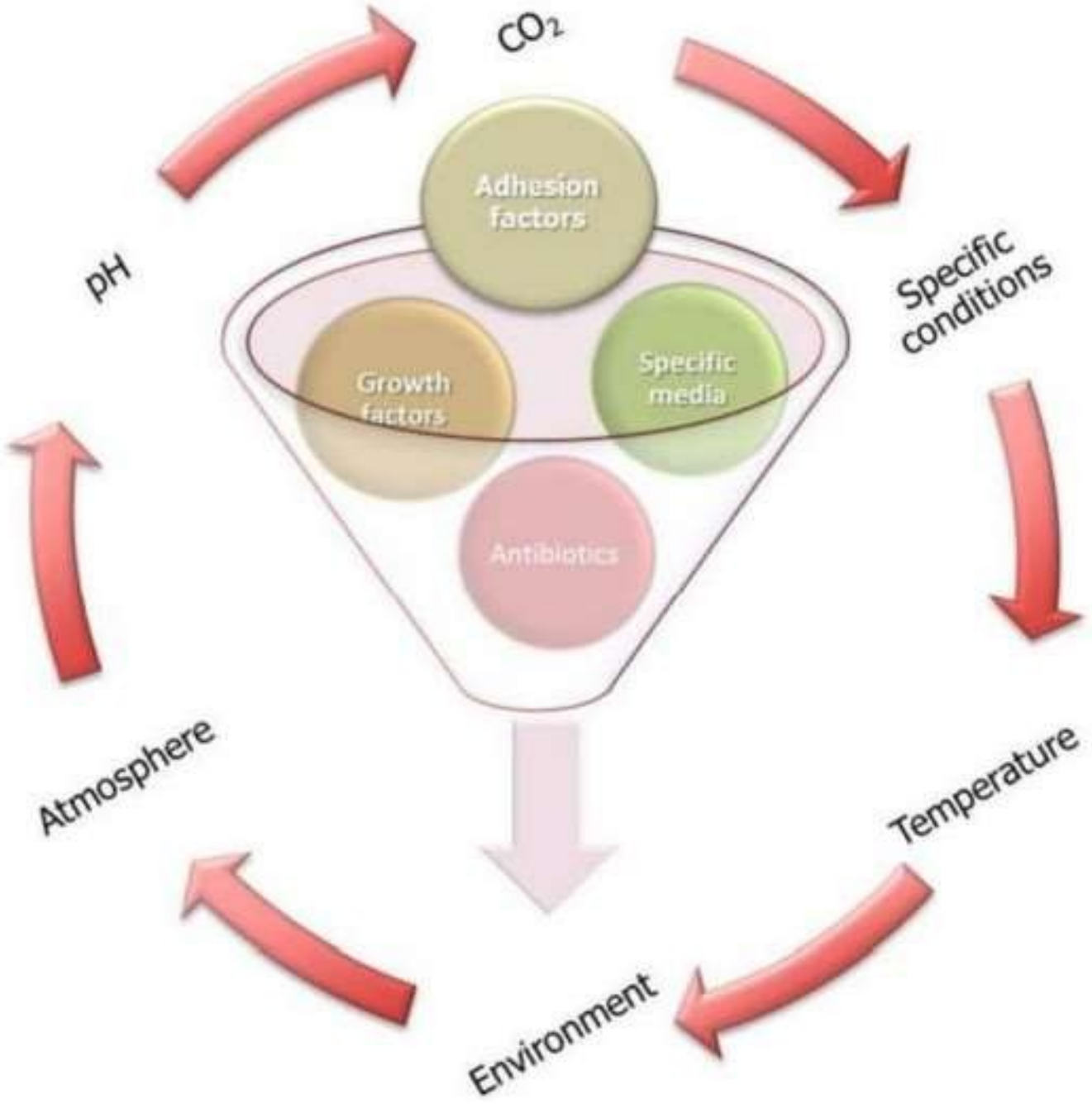
Why grow animal cells inculture?

- to investigate the normal physiology or biochemistry of cells. For example, metabolic pathways can be investigated by applying radioactively labeled substrates 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 inculture?

- 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.


A
B
C
D
E
Growth factors
Specific media
Antibiotics
Fetal Bovine Serum (FBS)


CO_2
Adhesion factors
Growth factors
Specific media
Antibiotics
Specific conditions
Temperature
Environment
Atmosphere
pH