Animal Tissue Culture

SQG 3242

Classification of animal cells and tissues

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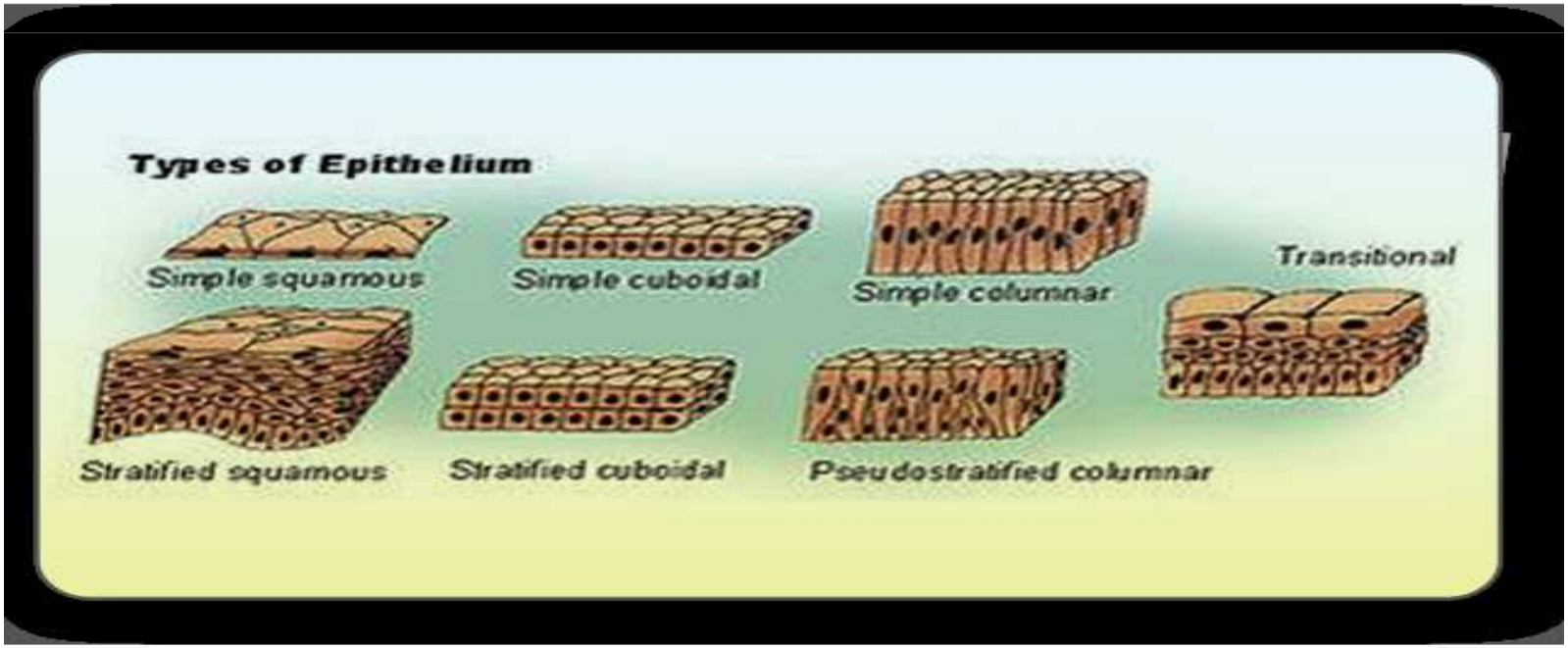
Classification of cell-types and tissue

- Epithelial tissue
- Muscle tissue
- Blood and lymph
- Connective tissue
- Nervous tissue

Epithelial tissue

- Form sheets that covering organ and lining activities
  - Skin
  - Linings of alimentary tract and lungs
- Classified according to their morphology



Connective tissue

- For the physical structure
  - Bone
  - Fibrous tissue
  - Cartilage
  - Tendon
- Having a specific ECM

CONNECTIVE TISSUE

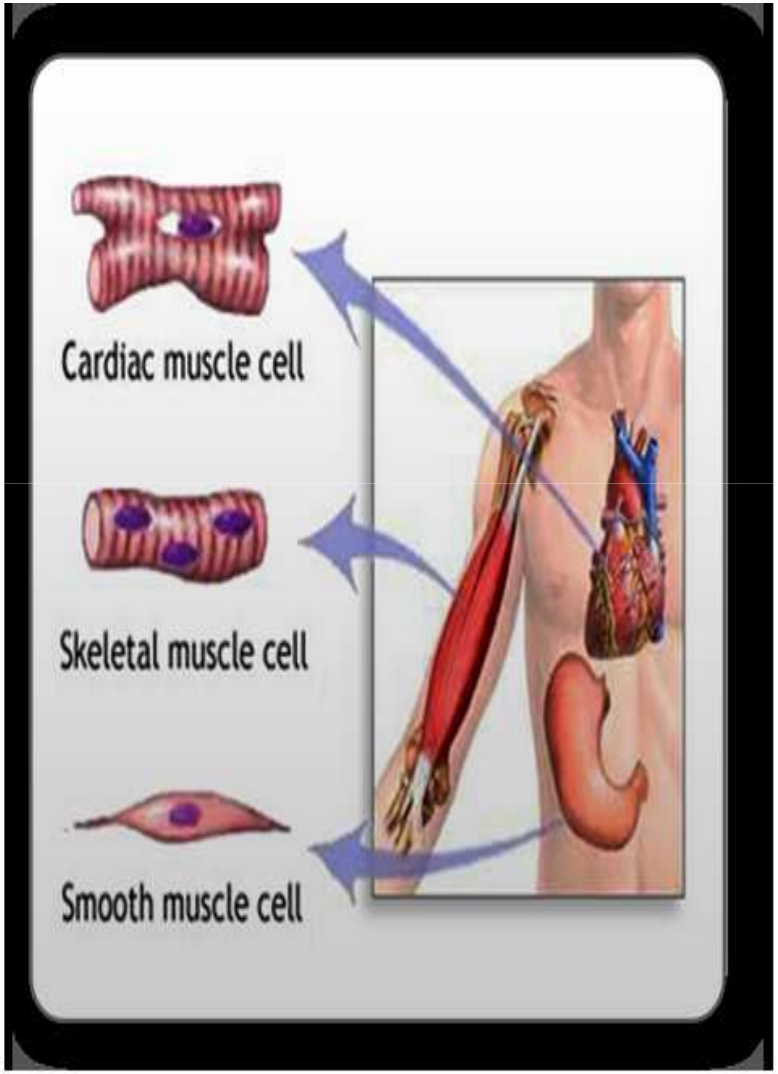
The spaces between organs and tissues in the body are filled with connective tissue made principally of a network of tough protein fibers embedded in a polysaccharide gel. This extracellular matrix is secreted mainly by fibroblasts.

fibroblasts in loose connective tissue

Two main types of extracellular protein fiber are collagen and elastin.

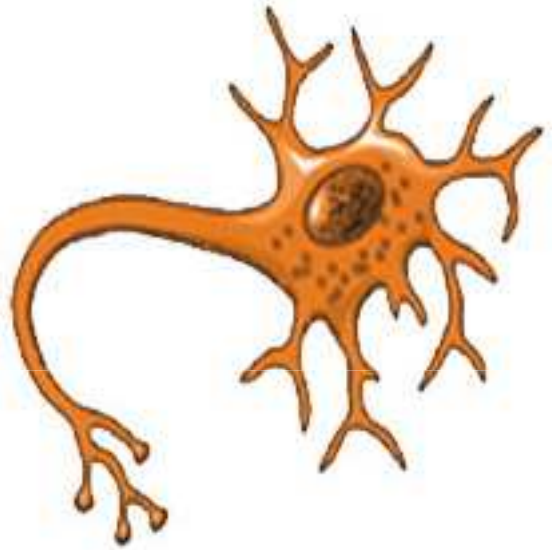
Muscle Tissue

- Consists of ordinary skeletal muscle, smooth muscle
- Occurs in
  - Intestine
  - Heart muscle

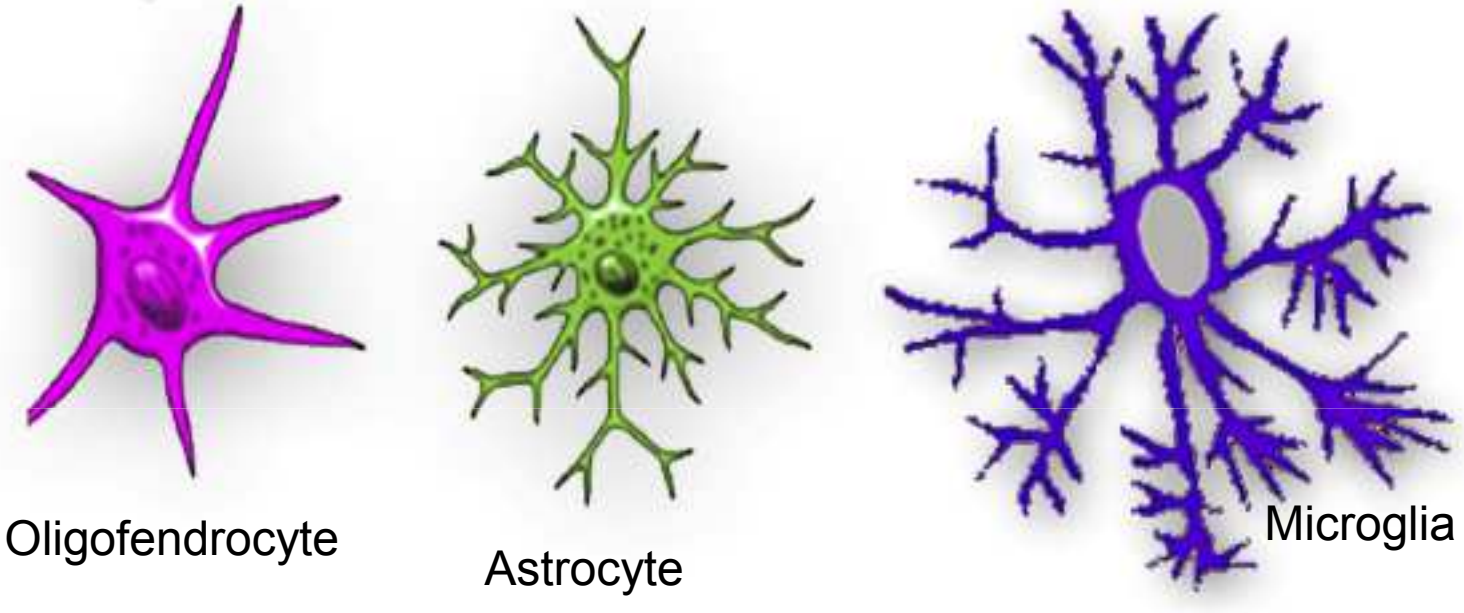


Nervous tissue

- Includes
  - Brain
  - Spinal cord
  - Peripheral nerves
  - Ganglia
- Highly complex



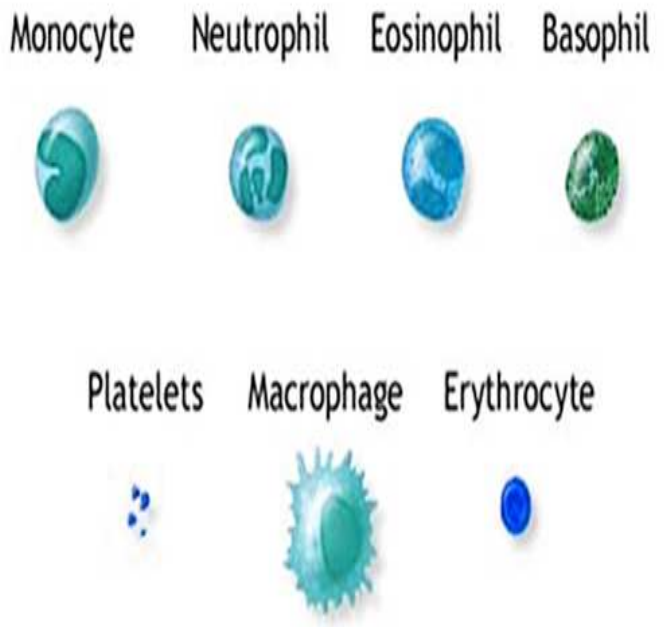
Structure of supporting cells in nervous system



- **Oligofendrocyte**– produce myelin to insulate the neuron and increase the action potentials
- **Astrocytes**– supporting cell – involved in metabolic exchange between neurons and blood
- **Microglia**–role in immune defence and can become a phagocytes –response to infections

Blood and lymph

- Include all the cells in the peripheral blood and their precursors in the bone marrow and lymph glands
- Structure:



Animal Cell and Tissue Culture

- Animal cell cultures are used as model systems for biochemical, physiological and pharmacological studies
- the production of growth factors, blood factors, monoclonal antibodies, interferons, enzymes, vaccines, and. hormones.

Cell type	Process investigated
Monocytes and macrophages	Pinocytosis and phagocytosis
Blood lymphocytes	Karyotype analysis for detection of genetic defects in humans
Normal and transformed fibroblasts	Surface adherence properties of normal and malignant cell membranes
Kidney tubule epithelial cells	Differentiation of monolayers; electrical and vectorial transport of solutes; monoclonal antibody production
Myeloma cells and ß-lympohcytes	Purification and characterization of specific membrane proteins e.g. α-and ß-adrenergic receptors, dopamine receptors
Kidney epithelial cells	To investigate relationship between membrane polarity and budding properties of envelope RNA viruses
Transformed leucocytes, fibroblasts and either lymphocytes or lymphoblastoid cells	Cells are infected with Sendai virus to produce Â, ß, and interferon respectively
Transformed Hela cells, mammalian cells	Radiation therapy and the design of radiosensitisers and radioprotectors
Mouse fibroblasts	Acute and chronic toxicity testing and metabolism of xenobiotics; vaccine production
Primary monkey kidney cells	Production of poliovaccines; hormone secretion
Fibroblasts, mammalian brain cells	To identify chemicals capable of including chromosome aneuploidy

Growing cells in culture

- The maintenance of cells outside of the living animal (in vitro) for easier experimental manipulation and regulation of controls.

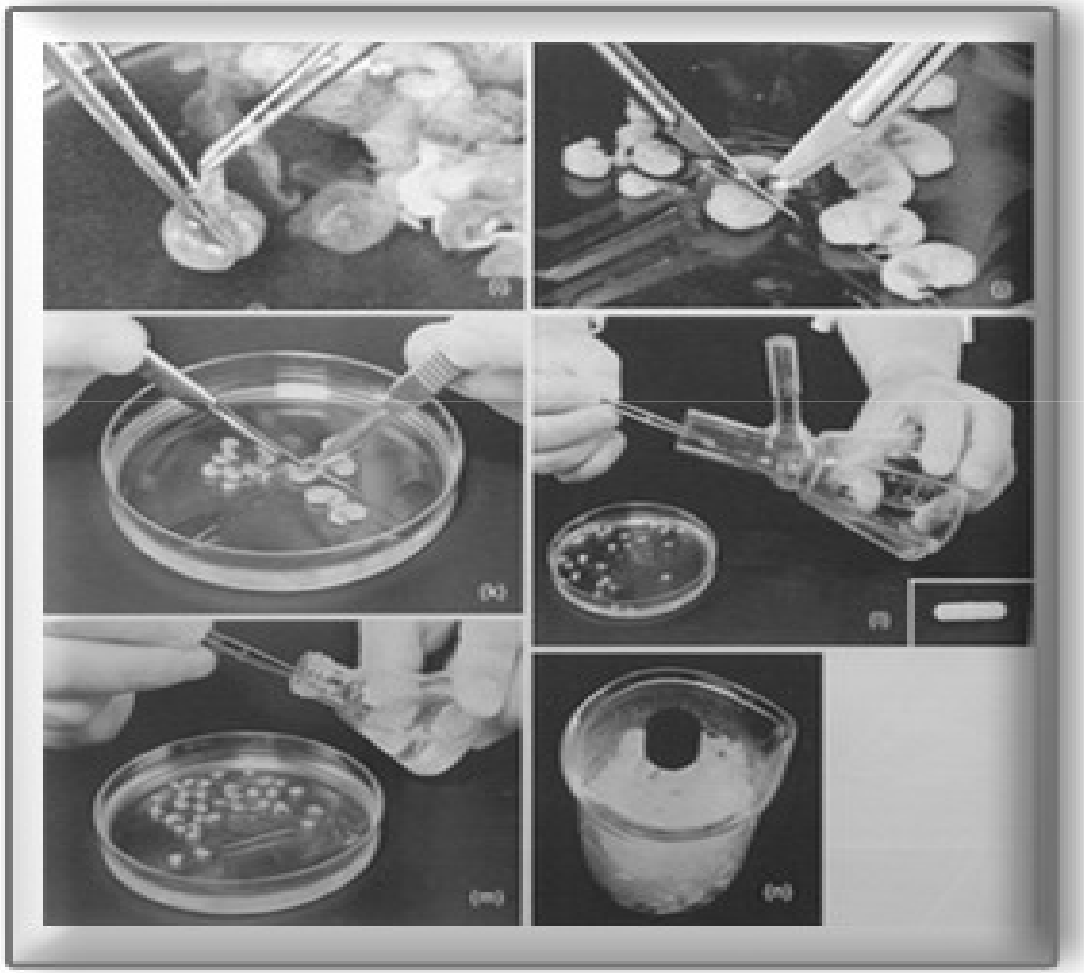
- **Pros**
- Use of **animals reduced**
- Cells from one cell line are homogenous and have same growth requirements, **optimizing growing patterns.**
- *In vitro* models allow for **control** of the **extracellular environment**
- Able **to monitor various elements** and secretions without interference from other biological molecules that occurs *in vivo*

- **Cons**
- Removal of cells from their ***in vivo*** **environment** means **removing** the cells, hormones, support structures and various other chemicals that the cells **interact with *in vivo***.
- It is nearly impossible to **recreate the *in vivo* environment**. The artificial conditions could cause cells to de-differentiate which will cause them to behave differently and produce proteins other than it would *in vivo.*
  - ***Genotype***: the genetic make-up of the cell
  - ***Phenotype***: the appearance and behavior of a cell as a result of their genotype. Most often, scientists are looking at phenotypic changes in their analysis of cells in culture

Primary culture

- Cells that are cultured directly from the subject
- With the exception of some derived from tumours, most primary cell cultures have limited lifespan.
- After a certain number of population doublings cells undergo the process of senescence and stop dividing, while generally retaining viability.
- An established or immortalised cell line has acquired the ability to proliferate indefinitely either through random mutation or deliberate modification, such as artificial expression of the telomerase gene.

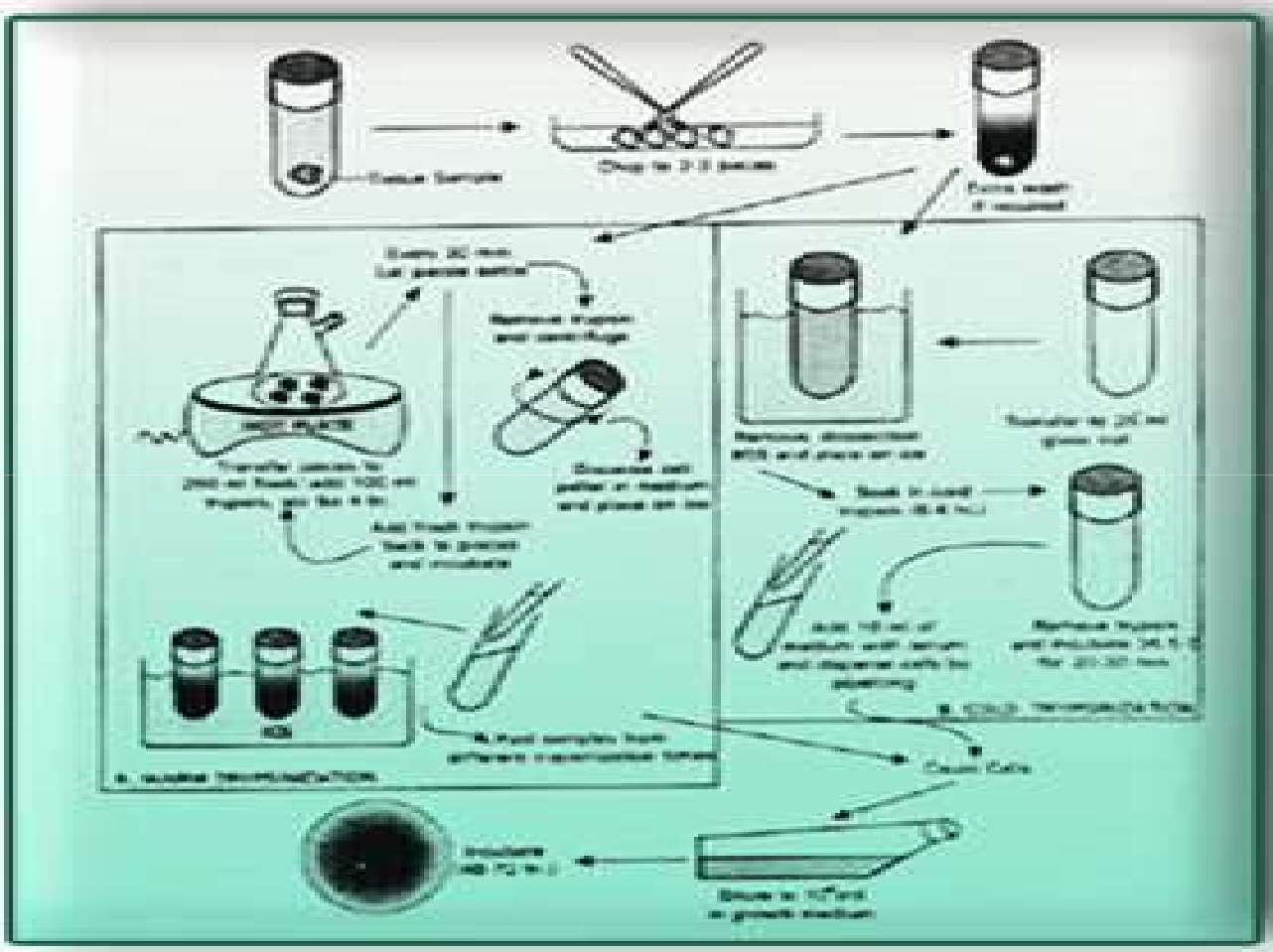
Making a Primary Culture



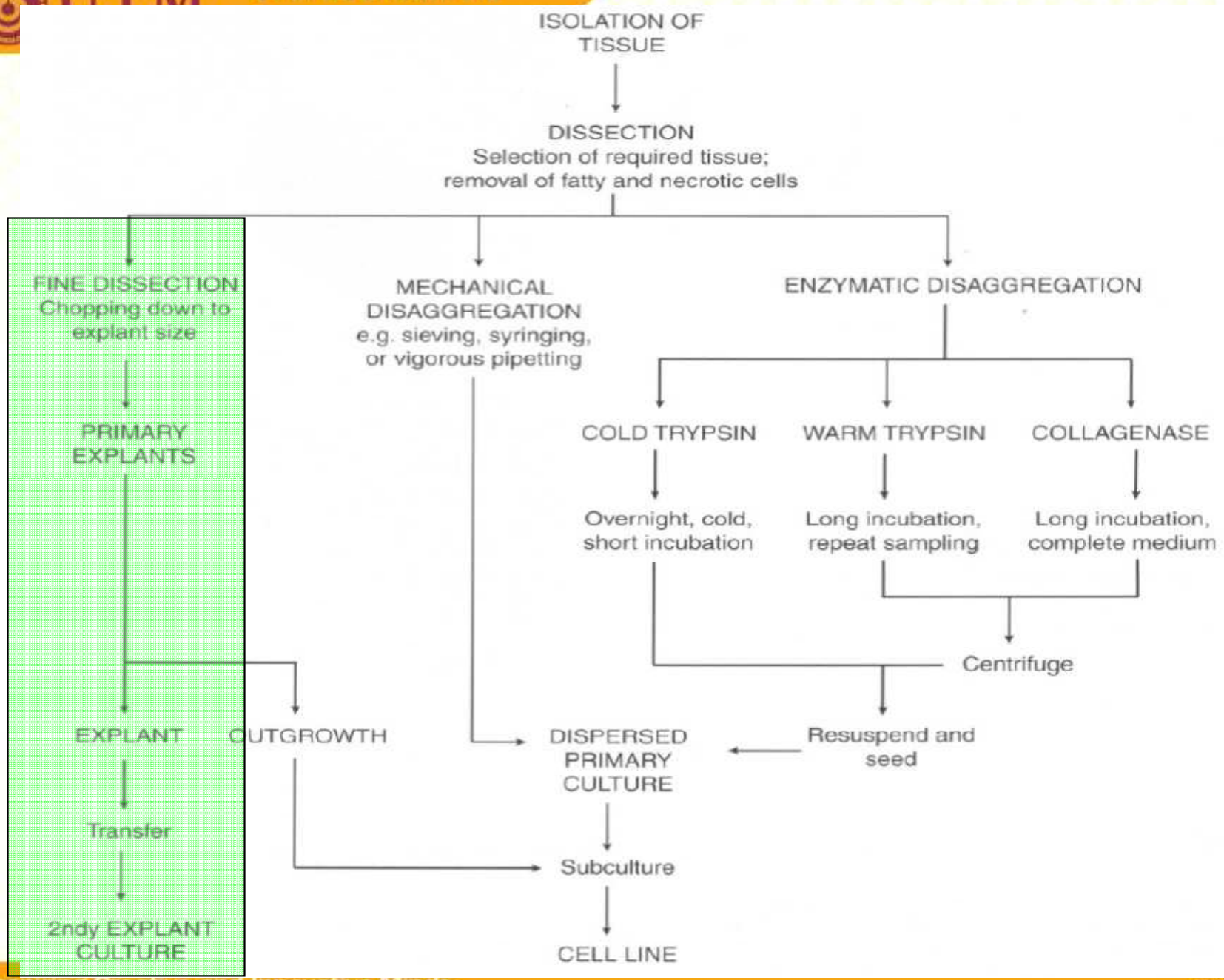
Isolation of free cells

- The major problem associated with the isolation of free cells and cell aggregates from organs is that of releasing the cells from their supporting matrix without affecting the integrity of the cell membrane.

- Various methods are employed to achieve this goal.



Isolation of free cells


ISOLATION OF TISSUE
DISSECTION
Selection of required tissue; removal of fatty and necrotic cells
FINE DISSECTION
Chopping down to explant size
MECHANICAL DISAGGREGATION
e.g. sieving, syringing, or vigorous pipetting
ENZYMATIC DISAGGREGATION
PRIMARY EXPLANTS
COLD TRYPSIN
WARM TRYPSIN
COLLAGENASE
Overnight, cold, short incubation
Long incubation, repeat sampling
Long incubation, complete medium
Centrifuge
EXPLANT
OUTGROWTH
DISPERSED PRIMARY CULTURE
Resuspend and seed
Transfer
Subculture
2ndy EXPLANT CULTURE
CELL LINE

Cell Lines

- **Cell Line**
  - Cells that have undergone a mutation and won't undergo apoptosis after a limited number of passages. They will grow indefinitely.

- **Transformed cell line**
  - A cell line that has been transformed by a tumor inducing virus or chemical. Can cause tumors if injected into animal.

- **Hybrid cell line (hybridoma)**
  - Two cell types fused together with characteristics of each

Animal cells are sub-divided in to

A. Anchorage dependent cells

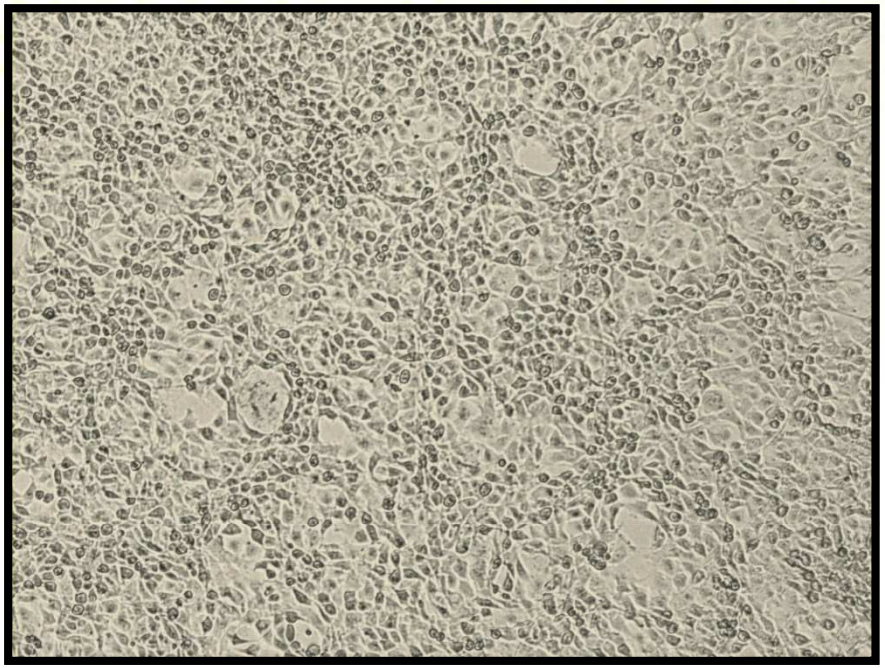
1. Those which remain viable only when attached to a solid substrate (e.g., primary cultures, normal diploid fibroblast cell strains, some established cell lines)

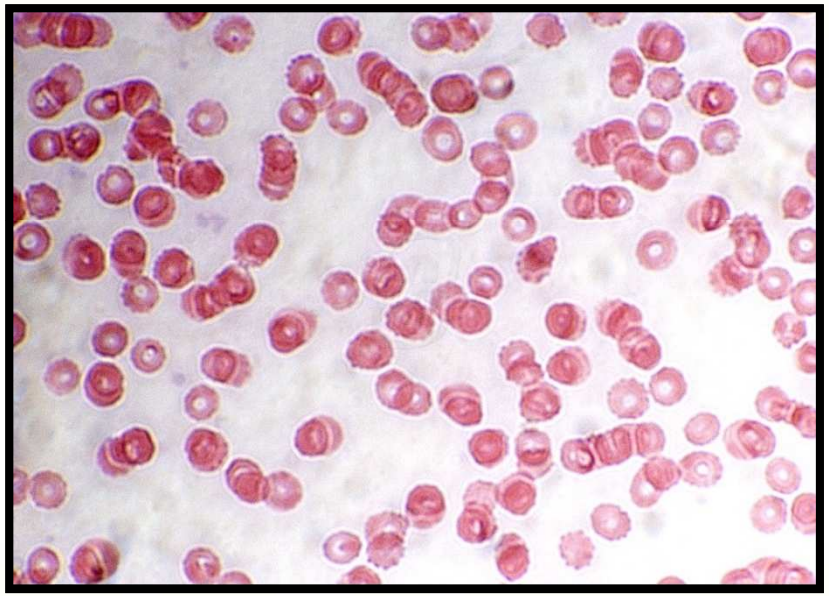
B. Anchorage independent cells

2. Those that will proliferate in fine suspension (e.g., murine leukemia cell line P388, Chinese hamster ovary -CHO cell lines).

Learn more from notes

Anchorage-Chondrocytes





Suspension-Red blood cells

Few popular cell lines

Continuous, from Normal Tissue							
293	Epithelial	Kidney	Human	Embryonic	Aneuploid	Readily transfected.	Graham et al., 1977
3T3-A31	Fibroblast		Mouse BALB/c	Embryonic	Aneuploid	Contact inhibited; readily transformed	Aaronson & Todaro 1968
3T3-L1	Fibroblast		Mouse Swiss	Embryonic	Aneuploid	Adipose differentiation	Green & Kehinde, 1974
BEAS-2B	Epithelial	Lung	Human	Adult			Reddel et al., 1988
BHK21-C13	Fibroblast	Kidney	Syrian hamster	Newborn	Aneuploid	Transformable by polyoma	Macpherson & Stoker, 1962
BRL 3A	Epithelial	Liver	Rat	Newborn		Produce IGF-2	Coon, 1968
C2	Fibroblastoid	Skeletal muscle	Mouse	Embryonic		Myotubes	Morgan et al., 1992
C7	Epithelioid	Hypothalamus	Mouse			Neurophysin;	De Vitry et al., 197
MDCK	Epithelial	Kidney	Dog	Adult	Diploid	Domes, transport	Gaush et al., 1966 Rindler et al., 1979
NRK49F	Fibroblast	Kidney	Rat	Adult	Aneuploid	Induction of suspension growth by TGF-α,β	De Larco & Todar 1978
STO	Fibroblast		Mouse	Embryonic	Aneuploid	Used as feeder layer for embryonal stem cells	Bernstein, 1975
Vero	Fibroblast	Kidney	Monkey	Adult	Aneuploid	Viral substrate and assay	Hopps et al., 196
Continuous, from Neoplastic Tissue							
A2780	Epithelial	Ovary	Human	Adult	Aneuploid	Chemosensitive with resistant variants	Tsuruo et al., 198
A549	Epithelial	Lung	Human	Adult	Aneuploid	Synthesizes surfactant	Giard et al., 1972
A9	Fibroblast	Subcutaneous	Mouse	Adult	Aneuploid	Derived from L929; Lacks HGPRT.	Littlefield, 1964b
B16	Fibroblastoid	Melanoma	Mouse	Adult	Aneuploid	Melanin	Nilos & Makarski 1978
C1300	Neuronal	Neuroblastoma	Rat	Adult	Aneuploid	Neurites	Liebermann & Sachs, 1978
C6	Fibroblastoid	Glioma	Rat	Newborn	Aneuploid	Glial fibrillary acidic protein, GPDH	Benda et al., 196

Maintaining cells in culture

- Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37°C, 5% CO_2) in a cell incubator.
- Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes being expressed
- Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the growth medium. Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrient components.
- The growth factors used to supplement media are often derived from animal blood, such as calf serum. These blood-derived ingredients pose the potential for contamination of derived pharmaceutical products with viruses or prions.

- Some cells naturally live without attaching to a surface, such as cells that exist in the bloodstream.
- Others require a surface, such as most cells derived from solid tissues.
- Cells grown unattached to a surface are referred to as suspension cultures.
- Other adherent cultures cells can be grown on tissue culture plastic, which may be coated with extracellular matrix components to increase its adhesion properties and provide other signals needed for growth

Animal Tissue Culture Media

Culture Media Containing Naturally occurring Ingredients

- The various kinds of such media used are:

(i) Blood plasma, (ii) blood serum, (iii) tissue extract and (iv) complex natural media.

(i) Blood plasma is used to provide:

1. Nutritive substrate and a supporting structure for many types of cultures, just as it also provides a matrix for new cells during the repair of injury in the body.
2. Conditioning the surface of glass for better attachment of cells.
3. Protecting cells and tissues from traumatic damage during subculture.
4. Protection from sudden changes in the environment at times of fluid change.
5. localized pockets of conditioned medium around cells.

Natural Media…

Blood Serum

- Blood serum *(plasma minus fibrinogen)* with or without other nutritive substances may be used either as the entire culture medium or as the fluid phase of a medium
- The importance of the low molecular weight growth factors provided by serum was understood later and even in a chemically defined simple medium like eagle's and dulbecco needs 10 to 20 % of serum supplement

Tissue Extracts

- Carrel (1912) discovered that embryo tissue extract had remarkable powers of promoting cell growth and multiplication in cultures of connective tissue cells from chick embryo heart.

Complex Natural Media

- Supplemented Hanks-Simms medium
- Supplemented bovine amniotic fluid medium
- Serum-supplemented yeast extract medium
- Serum-supplemented lactalbumin hydrolysate and yeast extract medium

Chemically defined media

- Earlier, the nutritive media for the cultivation of animal cells *in vitro* consisted of blood plasma, blood serum, tissue extracts, etc.
- The complexity and variability of these naturally occurring materials made it difficult to use
- attempts to devise chemically defined media were made by workers and many media were put to use
- Presently the constituents of cell culture media are all well defined and complex containing inorganic salts, amino acids, vitamins, glutamine, glucose and protein supplements and most cell cultures require a gas phase as well – O_2 and CO_2.
- Many media are made up of acid solutions and may incorporate a buffer.
- Each medium has a recommended bicarbonate concentration and CO_2 tension to achieve correct pH and osmolarity

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