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Faculty of Engineering & Technology

Medical Biotechnology MBT-413

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Introduction

- Cell culture can be defined as the process of cultivating cells and tissues outside the body of an organism*(invitro)* in an artificial environment, which stimulates the *invivo* conditions such as temperature, nutrition and protection from microorganisms.

- Cell culture was first successfully undertaken by Ross Harrison in 1907.

- Roux in 1885 for the first time maintained embryonic chick cells in a cell culture.

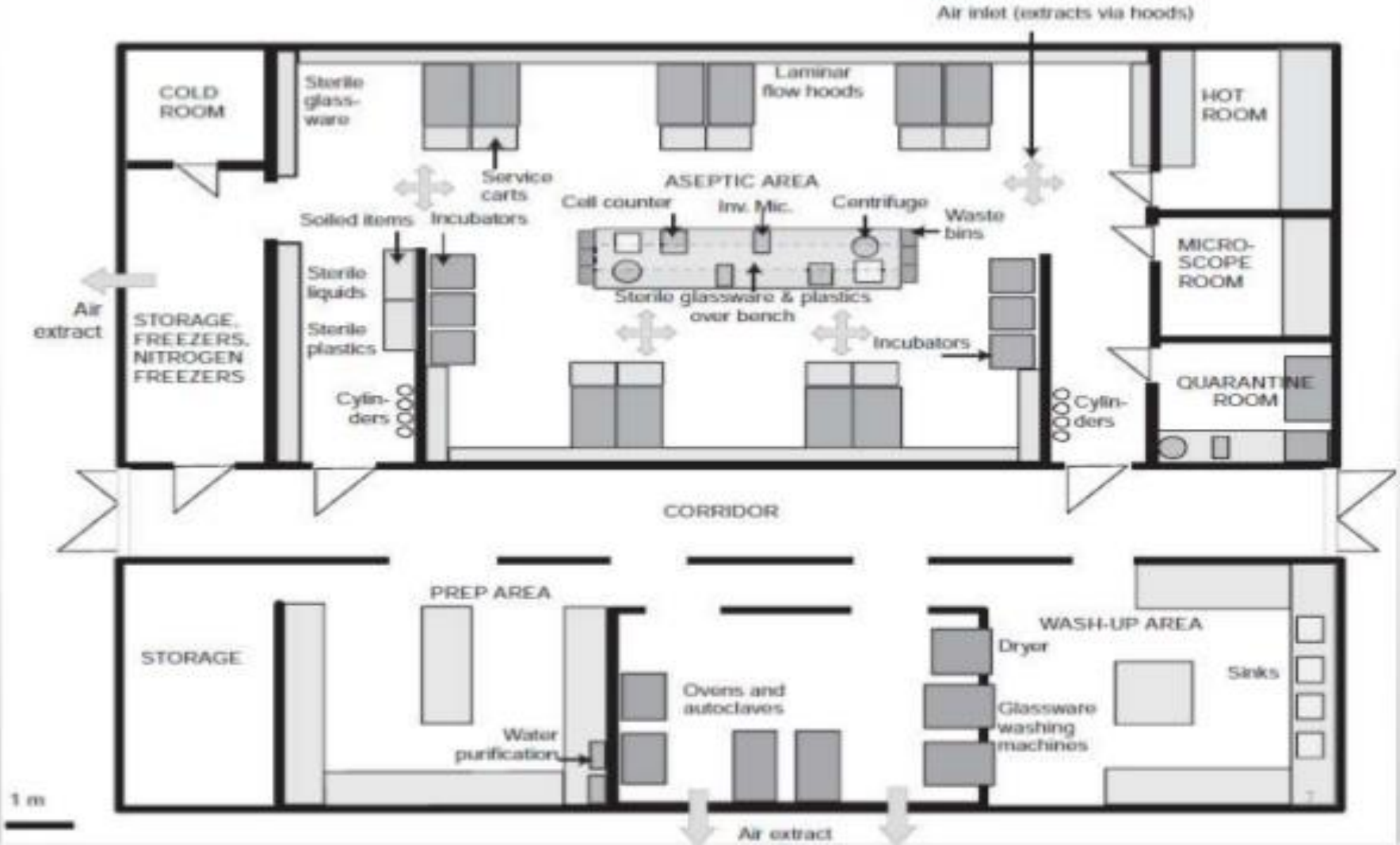
Major development's in cell culture technology

- 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.

Terminologies

- **Primary Cell Culture**: When cells are surgically removed from an organism and placed into a suitable culture environment they will attach, divide and grow.
- **Cell Line:** When the primary culture is subcultured and they show an ability to continuously propagate.
- **Anchorage dependency:** Cells grow as monolayers adhering to the substrate (glass/ plastic)
- **Passaging/ subculturing:** The process of splitting the cells.
- **Finite cells:** When the cells has finite life span.
- **Continuous cell lines:** When the cells can grow upto infinite lifespan.

Tissue culture Laboratory Design



Equipping Tissue Culture Laboratory

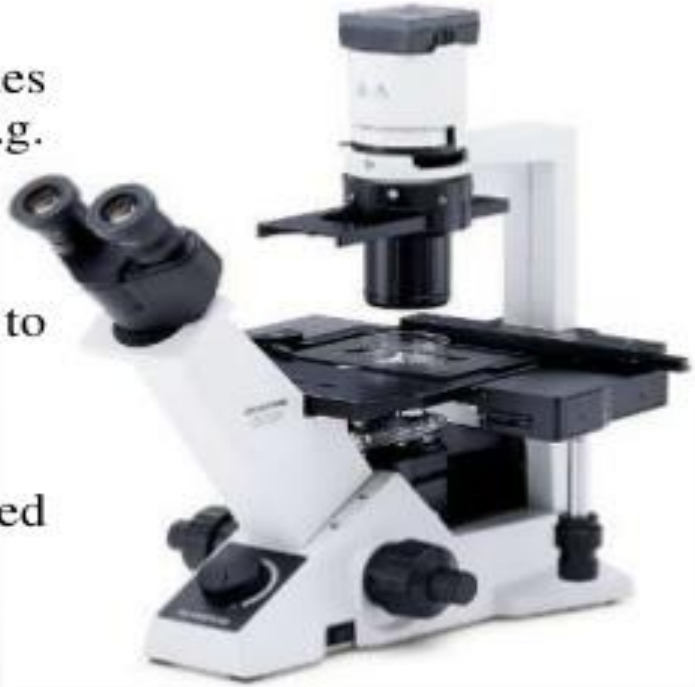
- Laminar cabinet - Vertical LAF are preferable. They are fitted with HEPA filters and UV.

- Incubation facilities - Temperature of 37°C, CO_2 2-5% & 95% air at 99% relative humidity.

- Refrigerators- Liquid media kept at 4°C, enzymes (e.g. trypsin) & media components (e.g. glutamine & serum) at -20°C.

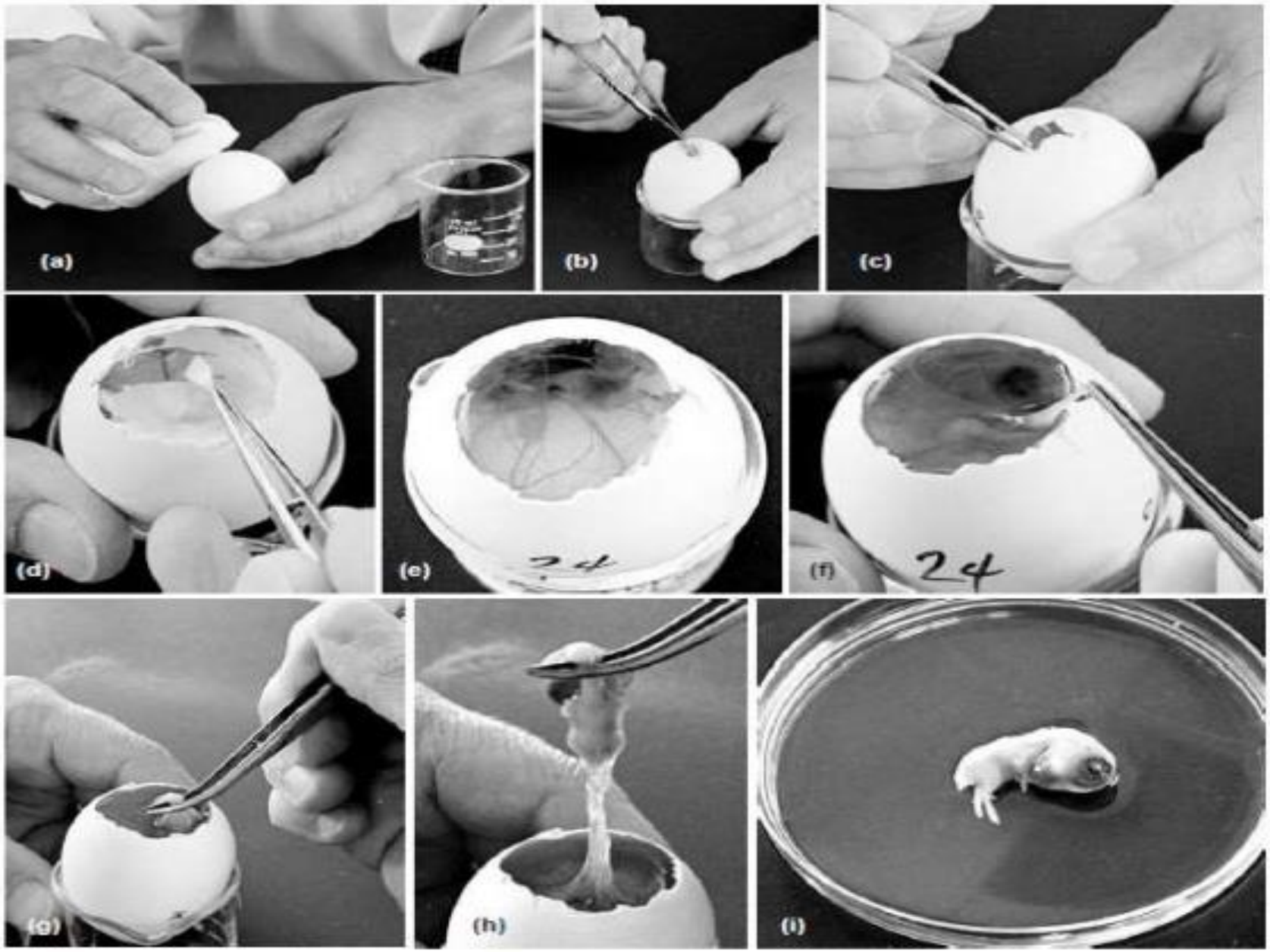
- Microscope- An inverted microscope with 10x to 100x magnification.

- Tissue culture ware- Culture plastic ware treated by polystyrene



Tissue Culture Media

- Cells have complex nutritional requirements that must be met to permit their propagation in vitro.
- Previously, scientists employed chick embryo extract, plasma, sera, lymph etc.,
- However, they varied in their growth promoting characteristics and thus hampering the reproducibility of the experiments.
- Today, a number of chemically-defined formulations have been developed that support the growth of a variety of established cell lines


(a)
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- Eagle's basal media
- Eagles's Minimum Essential Media (MEM)
- Dulbecco's Modified Essential Media (DMEM)
- Iscove's Modified Dulbecco's Medium (IMDM)
- Roosevelt Park Memorial Institute (RPMI 1640)
- HAM's F12

- The various nutrients required are:
  - glucose,
  - fats and fatty acids,
  - lipids, phospholipids and sulpholipids,
  - ATP and amino acids
  - Vitamins
  - Minerals
- Serum:

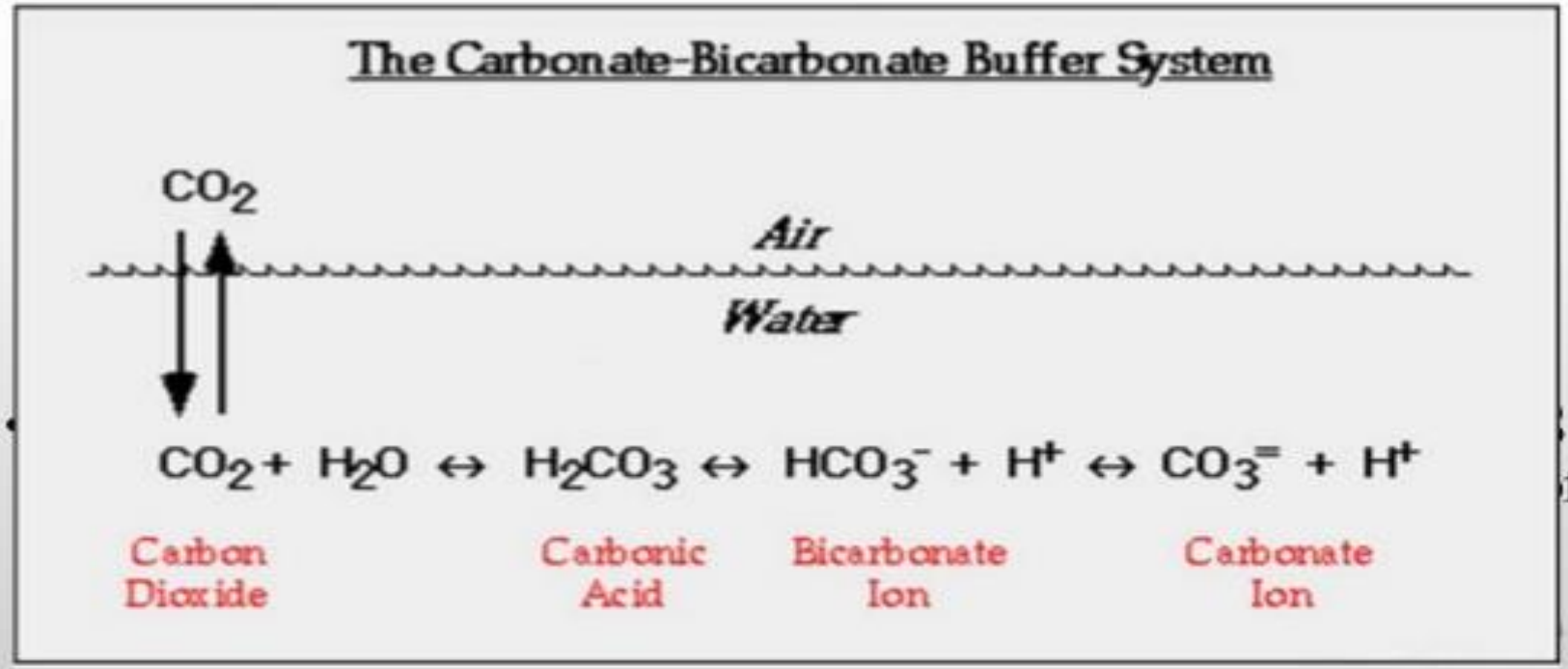
  Serum can provide various growth factors, hormones, cell adhesion factor and other factors needed by the most mammalian cells for their long term growth and metabolism.

  - FCS, FBS, CS, HS, HoS.

Properties and Special Requirements of Media

- **pH:** Optimum pH between 7.2 to 7.4 is generally needed for mammalian cells. Phenol red is used as an internal indicator.

pH	Colour of the medium
7.8	Pink / purple



- **Oxygen**
  - Cells depend upon glycolysis for the supply of O_2
  - Selenium controls O_2 diffusion
  - Glutathione acts as free radical scavenger
- **Temperature**
  - The optimum temperature of mammal is 37°C.
  - Change of ± 5°C is acceptable.
- **Humidity**
  - For cell growth 100% humidity is essential to reduce evaporation of the media.
- **Antibiotics**
  - penicillin (100 U/ml) for bacteria,
  - streptomycin (100 mg/ml) for bacteria,
  - or gentamycin (50mg/ ml) for bacteria,
  - and nystatin (50mg/ml) for fungi and yeast

Limitations of Serum

- It is undefined with respect to its chemical composition.
- It can be a source of adventitious agents and their by-products.
- Serum also presents a variable performance of cell growth and adds a substantial cost.
- Problem during downstream processing.
- Availability.

Serum Free Media

- Important amino acids, some trace elements, growth factor, hormone, transport protein and adhesion factor are added.
- Adhesion factor added are main components of intercellular substance and serum, such as fibronectin, collagen, and laminin.
- Primary purpose of introducing SFM is to promote the specific growth of a particular type of cell.

Types of tissue culture

- **CELL CULTURE**
- Tissue from an explant is dispersed, mostly enzymatically, into a cell suspension which may then be cultured as a monolayer or suspension culture

Advantages

- Development of a cell line over several generations
- Scale-up is possible
- Absolute control of physical environment
- Homogeneity of sample
- Less compound needed than in animal models

Disadvantages

- Cells may lose some differentiated characteristics.
- Hard to maintain
- Only grow small amount of tissue at high cost
- Dedifferentiation
- Instability, aneuploidy

- **EXPLANT CULTURE**
- Is the growth of tissues or cells separate from the organism.
- This is typically facilitated via use of a liquid, semi-solid, or solid growth medium, such as broth or agar.

Advantages

- Some normal functions may be maintained.
- Better than organ culture for scale-up but not ideal.

Disadvantages

- Original organization of tissue is lost.

ORGAN CULTURE

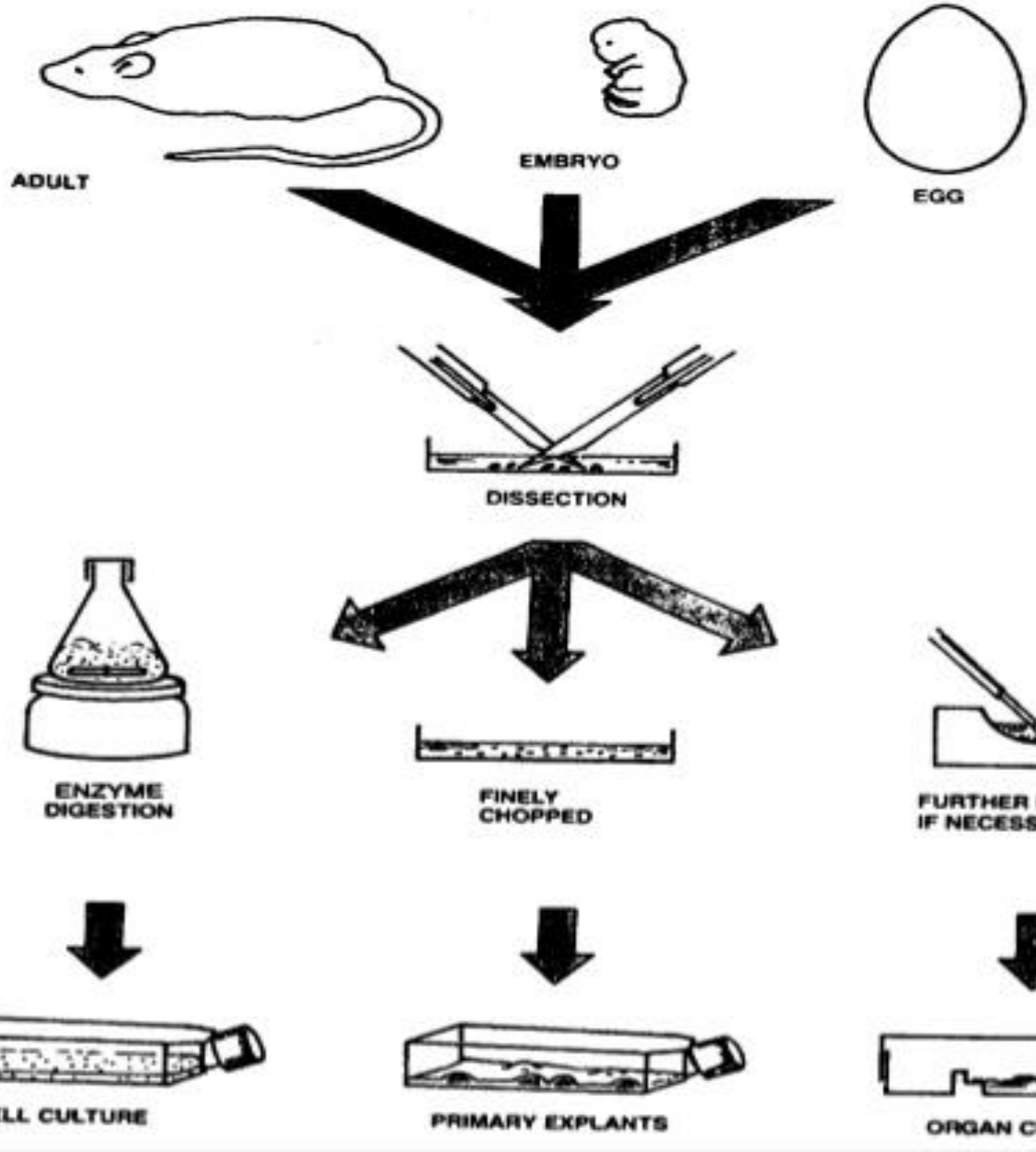
The entire embryos or organs are excised from the body and culture

Advantages

Normal physiological functions are maintained.
Cells remain fully differentiated.

Disadvantages

Scale-up is not recommended.
Growth is slow.
Fresh explantation is required for every experiment.


ADULT
EMBRYO
EGG
DISSECTION
ENZYME DIGESTION
FINELY CHOPPED
FURTHER DISSECTION IF NECESSARY
CELL CULTURE
PRIMARY EXPLANTS
ORGAN CULTURE

Primary Cultures

- When cells are surgically removed from an organism and placed into a suitable culture environment they will attach, divide and grow.

- Most of the primary culture cells have a finite lifespan of 50-60 divisions *invitro*.

- Primary cells are considered by many researchers to be more physiologically similar to in vivo cells

- Due to their limited lifespan, one cannot do long-term experiments with these cells

Tissue explants are excised using sharp scalpel.

↓

Mechanical disruption by pestle and mortar. Then filtered using a 0.22µ Filter fitted to a syringe.

↓

Enzymatic digestion by Trysin or collagenase

↓

Cells are counted on a Haemocytometer. 1-2 × 10^5 cells / mL is seeded in to the media.

↓

5mL of cells is suspended into 25cm^2 flask.

↓

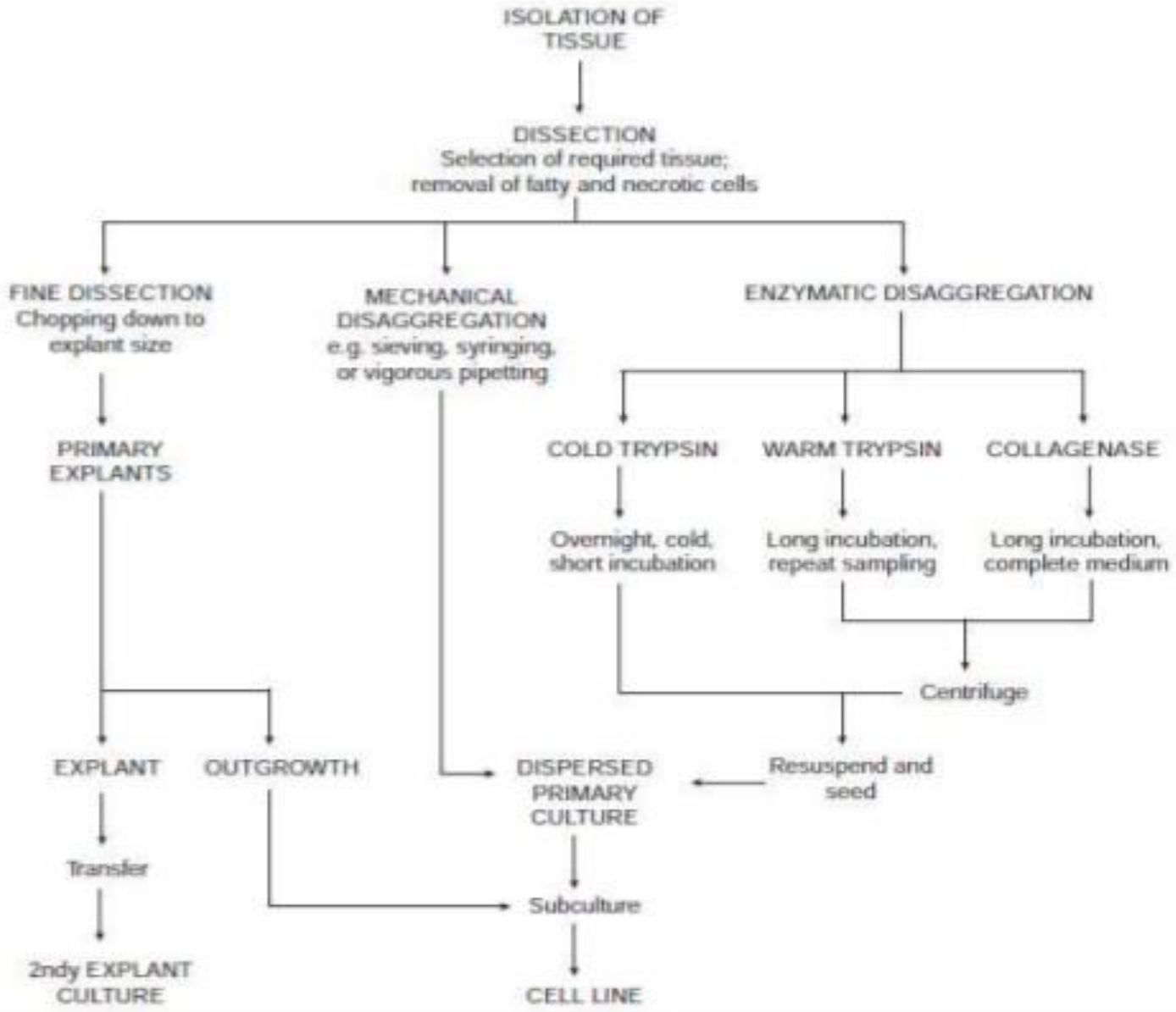
The flasks are incubated in a CO_2 **incubator**.

↓

The flasks are observed daily for their normal growth characteristics.

↓

Media is changed every 2-3 days until the cells attain 80% confluent.


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

Continuous cell lines

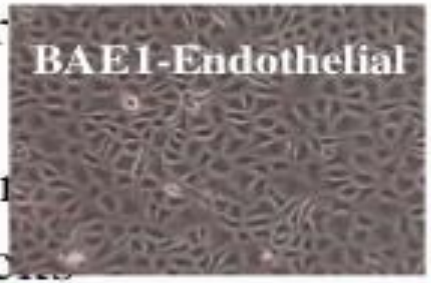
- Most cell lines grow for a limited number of generations after which they ceases.

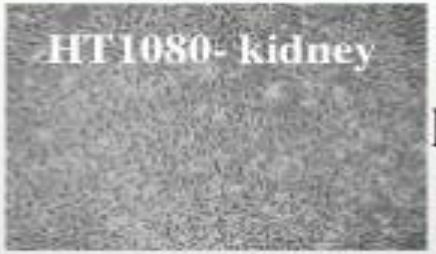
TABLE 12.1 Comparison of "Normal" and "Transformed" Cells

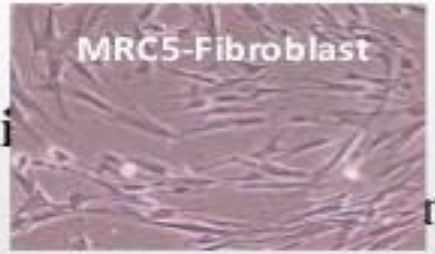
Normal	Transformed
1. Anchorage-dependent (except blood cells)	1. Nonanchorage-dependent (i.e., suspension culture possible)
2. Mortal; finite number of divisions	2. Immortal or continuous cell lines
3. Contact inhibition; monolayer culture	3. No contact inhibition; multilayer cultures
4. Dependent on external growth factor signals for proliferation	4. May not need an external source of growth factors
5. Greater retention of differentiated cellular function	5. Typically loss of differentiated cellular function
6. Display typical cell surface receptors	6. Cell surface receptor display may be altered

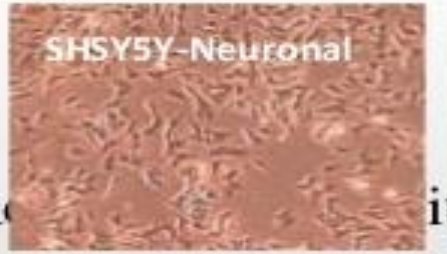
Common cell lines

Human cell lines









- Cos-7 African green monkey kidney cells
- And others such as CHO from hamster, sf9 & sf21 from insect cells

Subculturing

Remove spent media from the culture vessel.

Add the pre-warmed dissociation reagent such as trypsin. Gently rock the container to get complete coverage of the cell layer.

Incubate the culture vessel at room temperature for approximately 2 minutes.

Add equivalent of 2 volumes of pre-warmed complete growth medium. Disperse the medium by pipetting over the cell layer surface several times.

Then split the cells into 2 or 3 flasks containing complete media.

Incubate the cells.

Cryopreservation

Remove the growth medium, wash the cells by PBS and remove the PBS by aspiration.

Dissociate the cells by trypsin

Dilute the cells with growth medium.

Centrifuge at 200g for 5 min at RT and remove the growth medium by aspiration

Resuspend the cells in 1-2ml of freezing medium containing DMSO.

Transfer the cells to cryovials, incubate the cryovials at -80°C overnight

Next day transfer the cryovials to Liquid nitrogen.

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

Invitro Transformation of Cells

- Transformed, Infinite or Established Cells
- Changed from normal cells to cells with many of the properties of cancer cells
- Some of these cell lines have actually been derived from tumors or are transformed spontaneously in culture by mutations
- Chemical or gamma ray treated cells can become infinite with loss of growth factors
- Viral infection with SV40 T antigen can insert oncogenes and lead to gene alteration
- No matter how transformation occurred, the result is a cell with altered functional, morphological, and growth characteristics

Advantages of Tissue Culture

- ✓Control of the environment.
- ✓Characterization and Homogenity of sample.
- ✓Economy, Scale and Mechanization.
- ✓Invitro modelling of Invivo Conditions.

Limitations

- To grow cells outside their normal environment, three major controls are involved.
  - Observing scrict asepsis
  - Providing the right kind of physic-chemical environment
  - Nutrients in its simplest absorbable form
- Culturing technique needs a great deal of expertise.
- Tissue samples consists of a mixture of heterogenous cell populations
- Continuously growing cells often show genetic instability.
- Differences in the behavior or cells in cultured and in its natural form.
- Should include proper balance of the hormones.

Applications of Cell Culture

- **Excellent model systems for studying:**

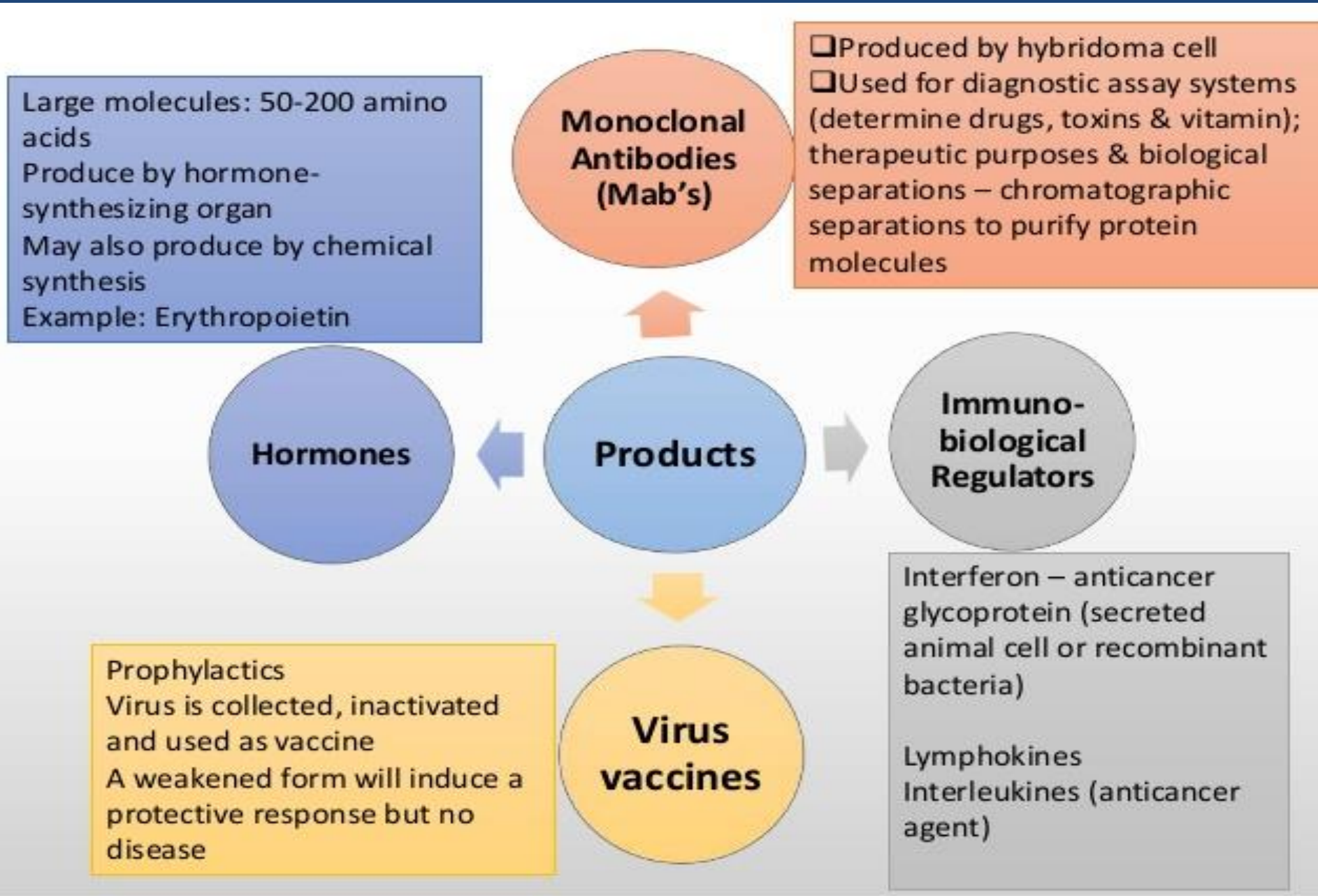
✓ The normal physiology, cell biology and biochemistry of cells

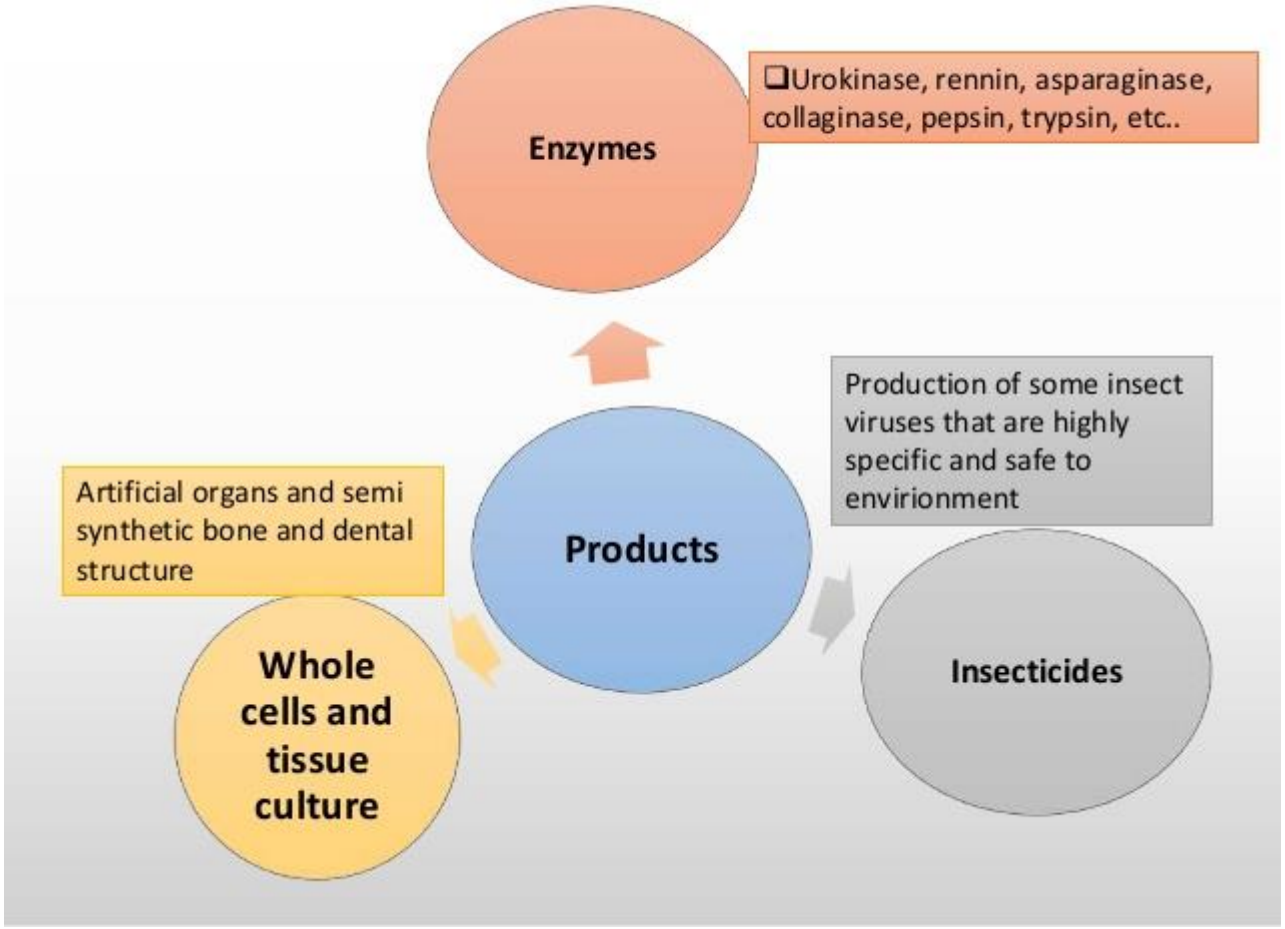
✓ The effects of drugs, radiation and toxic compounds on the cells

✓ Study mutagenesis and carcinogenesis

- **Used for gene transfer studies.**

- Large scale manufacturing of biological compounds
- (vaccines, insulin, interferon, other therapeutic protein)


Large molecules: 50-200 amino acids
Produce by hormone-synthesizing organ
May also produce by chemical synthesis
Example: Erythropoietin
Monoclonal Antibodies (Mab's)
❑Produced by hybridoma cell
❑Used for diagnostic assay systems (determine drugs, toxins & vitamin); therapeutic purposes & biological separations – chromatographic separations to purify protein molecules
Hormones
Products
Immuno-biological Regulators
Interferon – anticancer glycoprotein (secreted animal cell or recombinant bacteria)
Lymphokines
Interleukines (anticancer agent)
Prophylactics
Virus is collected, inactivated and used as vaccine
A weakened form will induce a protective response but no disease
Virus vaccines


Enzymes
❑Urokinase, rennin, asparaginase, collaginase, pepsin, trypsin, etc..
Production of some insect viruses that are highly specific and safe to envirionment
Artificial organs and semi synthetic bone and dental structure
Products
Whole cells and tissue culture
Insecticides