Animal Biotechnology
Lec. 3

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Technique of Cell Culture

- 1- Mechanical Technique: this includes the forcing of the tissue through cheese or silk cloth or shaking the tissue with beads in an appropriate buffer. This technique could results of cell damage and results in low cell yield.
- Biochemical Technique: this technique overcome the problem associated with mechanical one.
- Collagenase and hyaluronidase in a calcium free medium have been used for hepatocyte isolation. E.g. the liver is thinly sliced and then incubated with enzyme.

Types of animal Cells

- Those which remain viable only when attached to a solid substrate. (fibroblast cell strains)
- Those will proliferate in fine suspension (e.g. tumor cells, Hela cell lines)

Tissue culture Equipments

- Sterile handling: this includes a cell culture and manipulation area which should be adjacent to an incubation and storage area.
- Support service which include washing up, preparation and sterilization.
- The important consideration during culture is to minimize the chance of microbiological contamination.

Large Tissue Culture Equipments

A- Laminar flow cabinet: laminar flow is needed for culture aseptic technique.

- Class II laminar flow cabinet are needed to protect both the worker and the tissue culture.
- Laminar flow either contain HEPA (efficiency of 99.999%) or HPA filter.
- Laminar flow with Two HEPA filter: it has part-open fronted cabinets with double HEPA filter.

Large Tissue Culture Equipments

B- Incubators: provide controlled environmental conditions for cell culture which mimic conditioned experienced by cells *in vivo.*

- Incubators is a simple insulated metal box with a door and basic temperature controls to water-jacketed CO_2 incubators with sophisticated electronics controls.
- Controls ensure the condition within the incubator is maintained at a constant enhanced 5-10% CO_2.
- Because of the open vessel, the chamber has to be humidified to minimize fluid loss due to evaporation.

Cell Handling Equipment

- Inverted Microscope: used to check the cell culture.

Functions of the main ingredients culture media

1- Balanced Salt Solution (BSS): provide important inorganic salts.

- Also it contains sodium bicarbonate which in most cases acts as a buffer but also work as an important metabolite.

2- Amino Acids and Vitamins: these solution provide the selection of essential nutrient required by mammalian cells for growth and division.

3- Other ingredient such as energy source e.g. glucose, and an indicator to aid visual assessment of the pH of the medium, usually phenol red.

Functions of the main ingredients culture media

- 4- Buffers:
- Due to the consumption of the nutrients and waste production pH is easily changed.
- To avoid this change buffer system is used, which help in maintaining constant pH.
- The ideal buffer should have the following properties:

maintain a given pH in a particular medium.

I. Should not interfere with chemical or biochemical process of the culture.

II. To show no impedance of measurement or observation made on the system.

- Sodium bicarbonate/carbon dioxide buffer is one of the most widely used buffer.

Functions of the main ingredients culture media

- 5- Antibiotics:
- Antibiotics are added to most cells.
- Antibiotics reduce the incidence of opportunistic contamination microorganism.
- Penicillin and streptomycin are most widely used.

Animal Tissue Culture Media

- In spite of the vast use of chemically defined media in tissue culture, it is still necessary in most undertakings to depend on naturally occurring substances derived from the organism, for example:
  i. Blood plasma
  ii. Blood serum
  iii. Tissue extract
  iv. Complex natural media.

A- Blood Plasma

- The first tissue culture was done in 1907 by Harrison in clotted frog lymph.
- Plasma provide a complete nutrient in which cells could survive and multiply.
- Plasma is obtained by centrifugation of whole blood before coagulation

A- Blood Plasma

- Advantage of plasma membrane:
  i. Provide a nutrient substrate and supporting structure for many types of cultures.
  ii. Facilitate attachment of the cell to the surfaces of the culture vessel.
  iii. Protect the cells and tissue from excessive traumatic damage during subculture.
  iv. Protect the cells and tissue from sudden changes in the environment during fluid changes.

B- Blood Serum

- It was found that serum should be supplemented with 10-20% to provide completely adequate medium for the continuous propagation of established cell lines.

B- Blood Serum

- **Major functions of serum**
- Contain basic nutrients and enzymes:
  1. Supply of growth factors and hormones,
  2. Contains factors promoting attachment & spreading on artificial surfaces
  3. Protease inhibitors

B- Blood Serum

- Disadvantage of serum in the medium:
  1. Serum quality varies from batch to batch. Therefore, every batch of serum needs fresh testing.
  2. The demand of serum usually exceeds the supply.
  3. Serum is an obstacle to purification during down stream processing.
  4. Increase medium coast.
  5. Serum stimulate undesired growth or inhibit growth in other cases.

Serum Free medium

Advantage of serum free medium:

- It has ability to make medium selective for particular cell type.
- It has high degree of purity of reagent and water.
- It needs high degree of clean apparatus.