Animal Cell Culture

Introduction

- ACC - is the process of culture animal cells outside the tissue (*in vitro*) from which they were obtained. It will continue to grow if supplied with appropriate conditions and nutrients
- The culture process allow single cells to act as independent units, much like bacterium or fungus
- The cells are capable of dividing
- Animal cell culture was successfully established in 1907

Advantages

- Consistency and reproducibility of results
- Toxicological testing procedures are less expensive
- Reduced chance of contamination

Disadvantages

- Cell characteristic can change after a period of continuous growth

Types of Culture

1) Primary culture

- The cells directly taken from animal tissue (kidney, lung) and placed into a suitable culture environment
- Grow in a form of monolayer on support surface
- Also refer as anchorage-dependent
- Cell usually heterogeneous but still represent the parent cell type as well as in the expression of tissue specific properties

Two (**2**) basic methods to get primary culture:

1) **explant culture**

- remove tissue
- attach to a glass/culture vessel
- bath with culture medium
- after few days, cell will move from tissue explant out onto culture vessel surface or substrate
- cell will begin to grow and divide

2) **Enzymatic dissociation**

- speeds up the process by adding digesting (proteolytic) enzymes
- enzymes dissolve the cement holding the cell together
- this creates a suspension of single cells
- then placed into culture vessels containing culture medium
- cells will begin to grow and divide

2) Secondary culture

- A cell line obtained from the primary culture is known as the secondary culture
- The first passage (sub culturing) of the primary culture
- Cell line = cell population that can continue growing through many subcultures
- Removing (sub culturing) cells using enzyme
- Cell lines show many alterations from the primary cultures including change in morphology and chromosomal variation
- Non anchorage-dependent cell

Type of cell culture systems

1) **Anchorage dependent culture/monolayer culture system**

- Requirement of cells for a solid substratum for attachment before growth can occur.
- Grown in special tissue culture containers such as MD bottles, T- flasks, Roux bottles and Rollers.
- The choice of containers based on number of cells needed, the nature of the culture environment, cost and personal preference.

2) Suspension culture system/ nonanchorage dependent culture

-The cells are grown either :

a)In magnetically rotated spinner flasks or shaken flasks where the cells are kept actively suspended in the medium

b)In stationary culture vessels such as T-flasks and bottles where unable to attach firmly to the substrate

c)In mass cultivation, animal cells are grown in bioreactors almost similar to plant cells.

Technique of cultivating animal cells

- Excise tissues from specific organ of animals (lung, kidney) under aseptic conditions.
- Transfer tissues into a growth medium containing serum and antibiotics in small T-flasks.
- These cells form a primary culture that usually attach onto the glass surface of flask in monolayer form.
- The cells growing on support surfaces are known as anchorage-dependent cells.
- Some cells grown in suspension culture and are known to be non anchorage-dependent cells.
- Then a cell line appear from the primary culture and known as secondary culture.
- Remove cell from the surface of flasks using trypsin and add serum to the culture bottle.
- The serum containing suspension is then use to inoculate secondary cultures.
- Many secondary lines can be adapted to grow in suspension and are non anchorage dependent.

Culture condition

- Happy environment = allows cells to increase in number by undergoing cell division (mitosis).
- Provide the cells with appropriate temp, good substrate for attachment and proper culture medium.

Temperature

- Usually set at the same point as the body temp of the host from which the cell obtained
- Cold-blooded vertebrates – 18-25°C
- Mammalian cells – 36-37°C
- Temp maintained by use of carefully calibrated and frequently checked incubators

pH

- Most cells in culture grow best at pH 7.4
- Common used buffer bicarbonate-CO_2 or HEPES
- Keep the pH medium in a range 7-7.4
- When using bicarbonate-CO_2 buffer, need to regulate the amount of CO_2 dissolved in the medium
- Done by using an incubator with CO_2 control set to provide an atmosphere with between 2% and 10% CO_2.

Good substrate for attachment

- Anchorage-dependent cells require good substrate for attachment
- Commonly used substrate – glass and specially treated plastics
- Attachment factors – collagen, gelatin, fibronectin, laminin (used as substrate coating to improve growth & function of cells derived from brain, blood vessels)

- Culture medium used need to :

i) meet basic nutritional requirement of cells

ii) support growth of cells

iii) regulate the pH and osmolality

iv) provide essential gasses (O_2 & CO_2)

- **Food portion of culture medium consist of :**

a) Carbohydrates (glucose, fructose)

** provide an energy sources as well as a precursor for biosynthesis*

b) amino acids (Glutamine)

** as a sources of precursors for protein synthesis*

Glutamine is normally included at higher concentrations in order to act as a precursor for the TCA cycle intermediates. However, ammonia is formed from the metabolic breakdown of glutamine and can be inhibitory to growth in some cultures.

c) Vitamins & hormones

*are present at relatively low concentrations and are utilized as metabolic cofactors.

Helps regulate and control the cell's growth rate and functional characteristics

d) Salts

*are included so that the solution is isotonic and has no imbalances with the intracellular content

Helps regulate the flow of substances in and out of the cell

e) Phenol red

*usually added as a pH indicator of the medium and accounts for the color of culture media

f) Additional media supplements

- Serum – is a cell free-free liquid recovered from blood. Eg fetal bovine serum, calf serum, horse serum

  normally added to culture media to promote cell growth.

- Antibiotic – are often included in media for short-term cultures in order to reduce the risk of contamination

- Common features of animal cell bioreactor:

1) Reactor should be gently agitated and aerated. Agitation speed ≈20rpm.

   Bubble-column & airlift reactor operating at high aeration may cause damage of cells

2) Supply of CO_2-enriched air

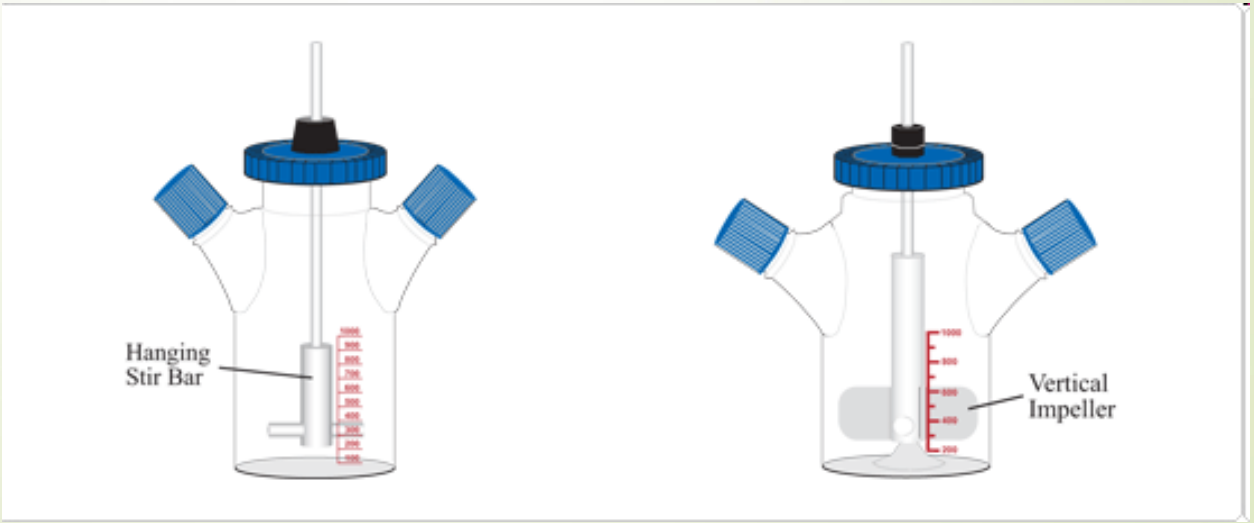
3) Removal of toxic products from metabolism eg lactic acid, ammonium

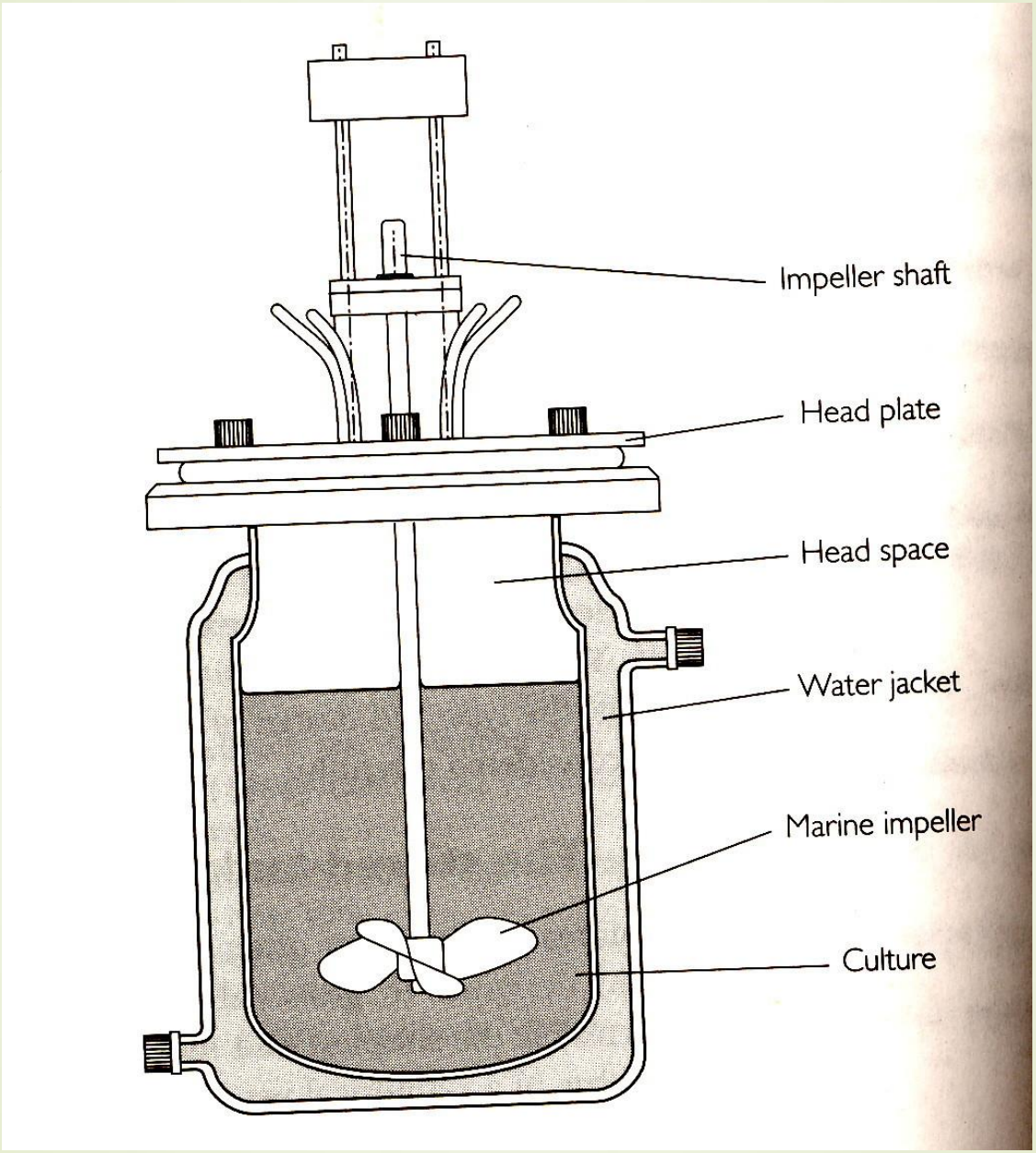
- Require gentler culture condition and control systems that are optimized for lower metabolic rates.
- Therefore, the design, mode of operation and control systems of Stirred Tank Reactor used for animal cells are distinctly different from those that would be applicable to bacterial or fungal cells.

- The fragility of animal cells culture has been a subject of considerable for fermenter design.
- Although cells in suspension can be damaged by various forces acting in a stirred culture, the major damaging force is from bubble bursting on the culture surface resulting from culture aeration.
- The simplest stirring operation involves the rotation of suspended bar by magnetic stirrer.
- This is the system used in glass spinner bottles and is suitable for stirring cultures up to 1 liter
- At larger volumes, magnetic stirrer are not suitable because of the increased energy required for rotation.
- In order to ensure adequate mixing at low strirring speeds, the culture vessels are designed with a round bottom, which distinguishes them from the flat-bottomed bacterial fermenter.
- Impeller blades which are fitted at the end of mechanical drive shafts are designed to allow vertical as well as horizontal liquid flow.

Magnetic stirrer for spinner bottles

Modified design of spinner bottles




Impeller shaft
Head plate
Head space
Water jacket
Marine impeller
Culture

Usage of Animal Cell Culture

1) **Model System**

- Provide a good model system for studying

  i) basic cell biology and biochemistry;

  ii) interactions between disease-causing agents and cells;

  iii) effects of drugs on cells; process and triggers for aging and nutritional studies.

2) **Toxicity Testing**

- Widely used to study the effects of new drugs, cosmetics and chemicals on survival and growth in wide variety of cell types.

3) **Cancer Research**

- To study differences in both normal cells and cancer cells.
- To study the mechanism of cancer with the use of use chemicals, viruses and radiation to convert normal cultured cells to cancer causing cells.

4) **Virology**

- One of the earliest and major uses of cell culture is the replication of viruses in cell cultures for use in vaccine production.
- Used in the clinical detection and isolation of viruses, as well as basic research into how they grow and infect organisms.

5) **Cell-Based Manufacturing**

- Three major areas cell-based industry are large-scale production of :

i) viruses for use in vaccine production (polio, rabies, chicken pox, hepatitis B and measles).

ii)cells that have been genetically engineered to produce proteins that have medicinal or commercial value (monoclonal antibodies, insulin, hormones).

iii)As replacement tissues and organs. Artificial skin for use intreating burns and ulcers is the first commercially available product.

- A potentially supply of replacement cells and tissues may come out of work currently being done with both embryonic and adult stem cells.

6) Genetic Counselling

- Amniocentesis, a diagnostic technique that enables doctors to remove and culture fetal cells from pregnant women. These cells can be examined for abnormalities in their chromosomes and genes.

7) Genetic Engineering

- To reprogram cultured cells with new genetic material (DNA and genes).
- Also can be used to produce new proteins in large quantity.

8) Gene Therapy

- The ability to genetically engineer cells has also led to their use for gene therapy.
- Cells can be removed from a patient lacking a functional gene and the missing or damaged gene can then be replaced.

9) Drug Screening and Development

- Cell-based assays have become increasingly important for the pharmaceutical industry as drugs.

Potential products from animal cultures

- Viral vaccines
- Monoclonal antibodies
- Recombinant proteins (glycoprotein)