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Animal Cell and Tissue Based Techniques

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Introduction

The term 'Tissue culture' refers to the culture of whole organism, tissue fragments as well as dispersed cell on a suitable nutrient medium. Tissue culture is divided into the two broad groups, i) cultures that facilitates cell to cell interactions and signaling among cell and allow their study and ii) those in which cell to cell interactions and signaling are missing. The first group consist of three distinct types of culture system, viz., (i) Organ culture (in this, whole embryonic organs or small tissue fragments are cultured *in vitro* in such a manner that they retain their tissue architecture, i.e., the characteristic distribution of various cell types in the given organ) (ii) Histotypic cultures (in this, individual cell lineages are first isolated from organ, purified and multiplied; they are grown separately to high density in three-dimensional matrix to study interactions and signaling among homologous cells. (iii) Organotypic cultures (in this, cells of different lineages are mixed together in specific ratios and spatial relationships in order to recreate a component of concerned organ). The second group consists of cell culture either as monolayer (cells are obtained either by enzymatic or mechanical dispersal of tissues into individual cells or by spontaneous migration of cells from an explants or as suspension culture.

Historical Background

The beginning of animal tissue culture can be traced to 1880 when Arnold showed that leucocytes can divide outside of body. Later in 1903, Jolly studied the behaviour of animal tissue explants immersed in serum, lymph or ascites fluid.

- 1907, **Harrison** – Frog embryo nerve fiber outgrowth *in vitro*. 1961, Hayflick & Moorhead- Definition of finite life span of normal human cells.
- 1912, **Carrel & Burrows** - Explants of chick connective tissues; heart muscle contractile for 2-3 months.

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- 1916, **Rous & Jones** - Trypsinization and subculture of explants.
- 1920, **Carrel & Ebeling** – Subculture of fibroblastic cell lines.
- 1954, **Abercrombie & Heaysman** - Fibroblast contact inhibition of cell mortality.
- 1961, **Hayflick & Moorhead** - Definition of finite life span of normal human cells.

Physiological conditions for the growth of cells

a) **pH** (potential of H^+ ion)

Optimum pH suitable for

Animal tissue – 7.4

Plant tissue – 5.8

Epidermal tissue – 5.5

Transformed tissue – 7.0 to 7.4

Fibroblast – 7.4 to 7.7

b) **Temperature :-**

Optimum temperature suitable for

Animal – 37°C

Birds – 38.5°C

c) **Gas Phase**

Two phases are required for *in vitro* growth of cells

- CO_2 - drops pH level

5% required by the cells

- O2 – 40 to 90% required

Some cells require more O_2, than extra O_2 carrier sources added *i.e.*, Hb

d) **Osmolarity** – Salt concentration of the cell

Human – 290 miliosmo/kg

Mice – 310 milliosmo/kg

e) **Foaming** – Characteristic of suspension

Drawbacks - Contamination

Denaturation of protein

Interfere with the exchange of gas phase

To prevent foaming add antifoaming agent *e.g.*, Silicon, Pluronic F68, CMC

Culture media

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The nutrient media for culture of animal cells and tissues must be able to support their survival as well as growth, *i.e.*, must provide nutritional, hormonal factors. The various type of media used for tissue culture may be grouped in 2 types (1) Natural and (2) Artificial. The choice of media depends upon the types of cell to be cultured and the objective of culture. The natural media is generally used for organ culture, while for cell cultures, artificial media with or without serum is used.

(A) Natural media: following are the types of natural media

- **Clots**: The most commonly used clots are plasma clot, which have been in use for a long time. It is now available in liquid and lyophilized state.
- **Biological fluids**: Of the various biological fluids used as a medium, serum is the most commonly used. Other mediums are Amniotic fluid, Ascitic fluid (aqueous humour from eye), Insect haemolymph.
- **Tissue extracts**: Chick embryo extract is the most used tissue extract, but bovine embryo extract is also used. Other tissue extract that have been used are spleen, liver, bone marrow, leucocytes etc

(B) Artificial media: Different artificial media have been used to serve one of the following purposes

- Immediate survival
- Prolonged survival
- Indefinite growth
- Specialized functions

There are main four types of artificial media available for cell culture

1) Serum containing media – EBSS, HBSS, PBSA
2) Serum free media – DME, Ham's F12, CMRL1066
3) Chemically defined media
4) Protein free media

Functions of serum

- It provides basic nutrient
- It provides hormones – e.g., insulin
- To supply the protein – e.g., fibronectin
- It provides binding protein – e.g., albumin

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- Increase the viscosity of medium
- It provides minerals
- Acts as a buffer

Disadvantages of serum

- Inhibits growth of some cells
- Contains come cytotoxic constituents – e.g., polyamine oxidase
- Variation in quality
- Supply is less than demand
- May be inadequate for some cell types

Advantages of serum free media

- Variation of serum is avoided
- Do not interfere while production of biochemicals
- Toxic effect is avoided
- No longer degradation of sensitive protein by serum
- Selective culture of differentiated cell from heterogeneous cultures

Disadvantages of serum free media

- Different media required for different cells
- Reliable serum free media are not available commercially
- Control of pH, temperature is required
- Growth rate & density is lower
- Cell becomes fragile

Major developments 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.

Initiation of cell culture

- Preparation and sterilization of substrate
- Preparation and sterilization of medium
- Isolation of explants

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- Disaggregation of explants (3 types, Mechanical, Enzymatic and EDTA treatment)
- Subculture
- Contamination

Cell Culture

Cell culture (a culture derived from dispersed cell taken from original tissue) may contain 3 types of cell

- Stem cells
- Precursor cells
- Differentiated cells

Types of cell cultures

1. Primary cell culture

- Freshly isolated cell cultures are called primary cell culture
- Heterogeneous and slow growing
- The primary cell culture could be of two types depending upon the kind of cells in culture

Anchorage Dependent/Adherent cells – Cells shown to require attachment for growth are set to be Anchorage Dependent cells. The Adherent cells are usually derived from tissues of organs such as kidney where they are immobile in connective tissue.

Suspension Culture/Anchorage Independent cells – Cells which do not require attachment for growth or do not attach to the surface of the culture vessels are anchorage independent cells/suspension cells. All suspension cultures are derived from cells of the blood system because these cells are also suspended in plasma in vitro e.g., lymphocytes.

2. Secondary cell cultures

- When a primary culture is sub-cultured, it becomes known as secondary culture or cell line. Subculture (or passage) refers to the transfer of cells from one culture vessel to another culture vessel.
- Sub-culturing – Sub-culturing or splitting cells is required to periodically provide fresh nutrients and growing space for continuously growing cell lines.
- The process involves removing the growth media, washing the plate, disassociating the adhered cells, usually enzymatically. Such cultures may be called secondary cultures.

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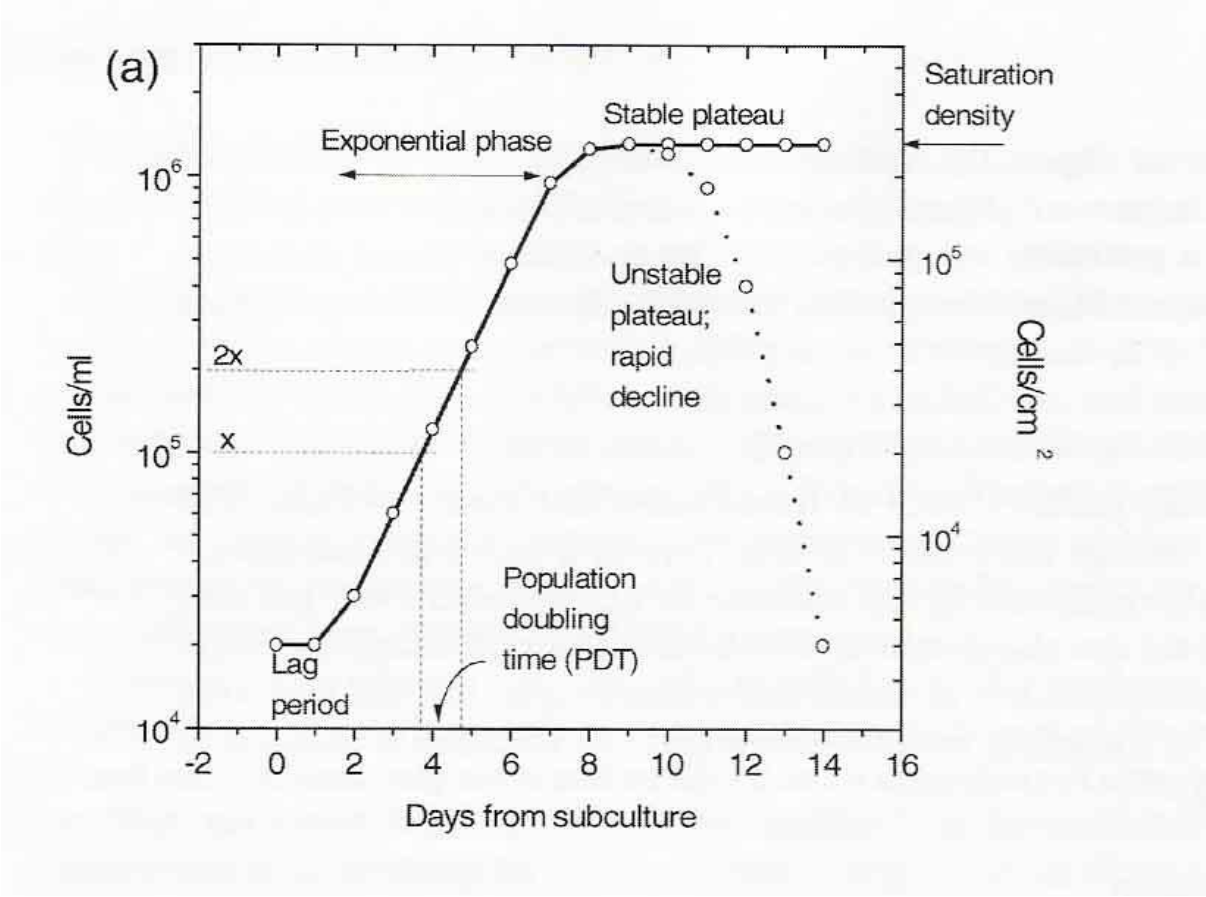
3. Monolayer cultures

- When the bottom of the culture vessel is covered with a continuous layer of cells, usually one cell in thickness, they are referred to as monolayer cultures.

4. Suspension cultures

- Majority of continuous cell lines grow as monolayer. Some of the cells which are non-adhesive e.g., cells of leukaemia or certain cells which can be mechanically kept in suspension, can be propagated in suspension.
- Cells of certain types can be grown in liquid media as suspension culture.
- Cells are harvested from late log phase cultures are inoculated into fresh liquid medium maintained at 37° C.
- The initial cell density is usually between 5-20 × 10^4 cells / ml.
- The cultures are stirred at 200-350 rpm.
- Growth curve follow the typical sigmoidal pattern; a lag phase of 2-24 hr followed by a log phase and culminating in a stationary phase; finally, the cells began to die.



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Sr. No.	Cell line	Product
1	Human tumour	Angiogenic factor
2	Human leucocytes	Interferon
3	Mouse fibroblasts	Interferon
4	Human Kidney	Urokinase
5	Transformed human kidney cell line, TCL-598	Single chain urokinase - type plasminogen activator (scu-PA)
6	Human kidney cell (293)	Human protein (HPC)
7	Dog kidney	Canine distemper vaccine
8	Cow kidney	Foot and Mouth disease (FMD) vaccine
9	Chick embryo fluid	Vaccines for influenza, measles and mumps
10	Duck embryo fluid	Vaccines for rabies and rubella
11	Chinese hamster ovary (CHO) cells	Tissue-type plasminogen activator (t-PA) β and gamma interferons Factor VIII

Organ Culture

- Definition – 3 dimensional cultures of un-disaggregated tissues.
- In vitro culture and growth of organs or parts thereof in which their various tissue components, e.g., parenchyma and stroma, are preserved both in terms of their structure and function so that the cultured organs resemble closely the concerned organs in vivo is called Organ Culture.
- The first attempt at organ culture was by Loeb in 1897, who maintained adult rabbit kidney, liver and ovary on a small plasma clot in test tube.

Techniques:

1) **Plasma clot:** In this method, the explants is cultured on the surface of a clot consisting of chick plasma and chick embryo extract contained in watch glass, so it is also called as watch glass technique.
2) **Raft methods:** In this approach, the explant is placed onto a raft of lens paper or rayon acetate which is floated on serum in watch glass. Rayon acetate raft were made to float on

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the serum by treating their 4 corners with silicon. Similarly, floatability of lens paper is enhanced by treating it with silicon.

3) **Agar Gel:** In this method, the medium is gelled with 1 % agar. This method avoids immersion of explants into the medium and permits the use of defined media.
4) **Gris method:** This method utilizes 25 mm × 25 mm pieces of a suitable wire mesh or perforated ss sheet whose edges are bent to form 4 legs of about 4mm height. Tissues are first placed on raft, which are then kept on the grid. The grids are then placed in a culture chamber filled with fluid medium up to the grid; the chamber is supplied with a mixture of O2 and CO2 to meet the high requirement of adult mammalian organs.
5) **Cyclic Exposure to Medium and Gas phase:** This technique has been successfully used in long term culture. The explants are intermittently exposed to the fluid medium and gas phase. The number of explants varies from 2-18 depending upon the organ cultured. The explants are attached to the bottom of a plastic culture dish and are covered with fluid medium. The dishes are closed in a chamber containing a suitable gas mixture and mounted on a rocker platform.

Advantages of Organ Culture

- The explants remain comparable to the in vitro organs both in structure & function which makes them more suitable than cell culture for physiological studies.
- The development of foetal organs *in vitro* is comparable to that *in vivo*. Hormone dependent organ remains so, while endocrine organs secrete the specific hormones.
- Organ culture provides information on patterns of growth, differentiation and development and influence of various factors on these features.
- Organ culture may replace whole animals in experimentation as the results from them are easier to interpret**.**

Application of Organ Culture

- Studies on pattern of growth, differentiation and development of organ rudiment *i.e.*, foetal organ, and the influence of various factors like hormones, vitamins etc. on these parameters
- The action of drugs, carcinogenic agents, etc on the animal organ is studied in vitro to at least serve as a guide for events in whole animals

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- To produce the tissue for the implantation in a patient, this is often called as tissue engineering, e.g., artificial skin

Limitations of Organ Culture

- Results from organ culture are often not comparable to those from whole organism studies, e.g., in studies on drugs action, since the drugs are metabolizing *in vivo* but not *in vitro*
- Organ culture can be maintained for a few months. But it may be desirable to study the effects of certain factors for several months. In such cases, the organs treated *in vitro* may be transplanted into suitable host animals, e.g., nude mice
- Organ culture is not suitable for biochemical and molecular analysis
- For each study, fresh organ culture has to be maintained, which makes them labour intensive
- Analysis of organ culture is largely based on histological sectioning for histochemical, immune cyto chemical and autoradiograph studies *in situ*

Applications

- To produce the antiviral vaccines and understanding of neoplasia
- To produce large number of cells suitable for biochemical analysis
- To try the preclinical analysis of newly synthesized pharmaceutical drugs
- To produce a human growth factors e.g., Insulin
- To study the cell interactions and intercellular control mechanism in cell development and differentiation
- To reveal genetic disorders in unborn child
- To determine the quality of drinking water
- Tissue engineering

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