CELL CULTURE TECHNOLOGY

SQG 3242

INTRODUCTION TO ANIMAL CELL CULTURE

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WHAT IS ANIMAL TISSUE CULTURE?

Introduction

Historical Background

- Derived in the beginning of twentieth century -method for studying the behavior of animal cells.
- It was elaborated first with undisaggregated fragment of tissue, the growth was restricted by needing the migration of cell from tissue fragment, with occasional mitoses in the outgrowth.
- Animal culture was first successfully undertaken by Ross Harrison in 1907- Frog embryo nerve fiber outgrowth *in vitro*
- In 1912, Alexis Carrel attempted to improve the animal cell culture with experiments on the culture of chick embryo tissue
- Since this kind of culture dominated the field for more than 50 years the name “Tissue Culture” stuck, in spite of the fact that most of the explosive expansion in this area since 1950, has utilized dispersed cell cultures

- Initial work was centered on cold-blooded animals then into warm-blooded animals.
- The embryonated egg was a favorite choice, but the development of experimental animal husbandry brought mammals to the forefront as favorite material.
- The demonstration that human tumors could give rise to cell lines interest → in human tissues.
- This was followed by a series of developments that made cell culture widely available tool for scientists- (development of antibiotics, trypsin to detach cells from culture vessels, chemically defined culture media that made far easier to grow cells).
- Cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. Viruses that growth in cell cultures allowed preparation of purified viruses for the manufacture of vaccines.

Terms

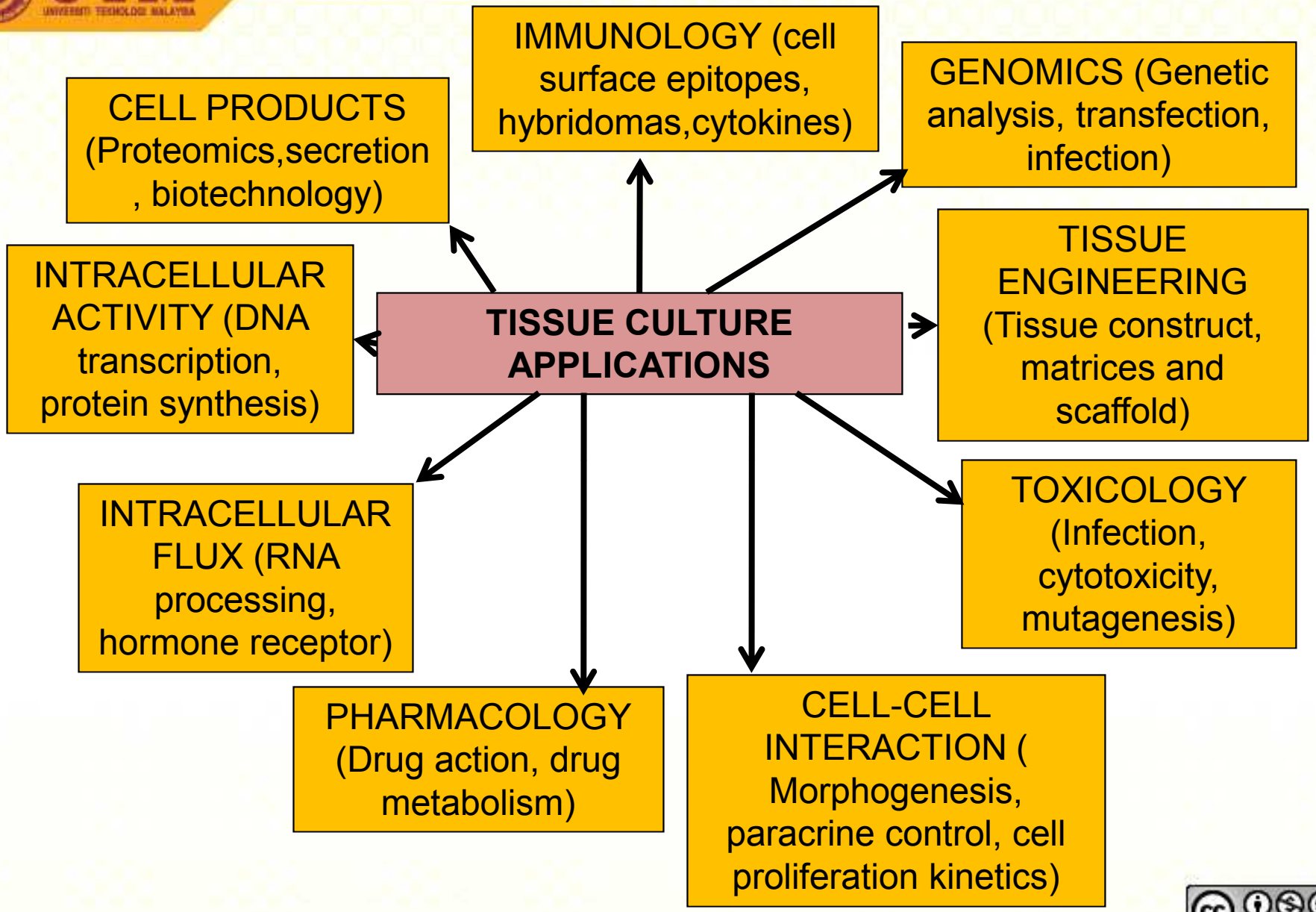
Tissue culture
(generic term to include organ culture and cell culture.)

Organ culture
a three-dimensional culture of undisaggregated tissue retaining some or all of the histological features of the tissue *in vivo*

Cell culture
a culture derived from dispersed cells taken from original tissue, from a primary culture, or from a cell line or cell stain by enzymatic,mechanical or chemical disaggregation

Histotypic culture – cells have been reaggregated or grown to re-create a three-dimensional structure with tissue-like cell density.

Organotypic – implies the same procedure but recombining cells of different lineages.


IMMUNOLOGY (cell surface epitopes, hybridomas,cytokines)
GENOMICS (Genetic analysis, transfection, infection)
CELL PRODUCTS (Proteomics,secretion , biotechnology)
INTRACELLULAR ACTIVITY (DNA transcription, protein synthesis)
TISSUE CULTURE APPLICATIONS
TISSUE ENGINEERING (Tissue construct, matrices and scaffold)
INTRACELLULAR FLUX (RNA processing, hormone receptor)
TOXICOLOGY (Infection, cytotoxicity, mutagenesis)
PHARMACOLOGY (Drug action, drug metabolism)
CELL-CELL INTERACTION (Morphogenesis, paracrine control, cell proliferation kinetics)

Advantages

- Behaviour of cells in culture is easily interpreted and regulated properly.
- There remains homogeneity of cell types (achieved through serial passages)
- The legal, moral and ethical questions of animal experimentation can be avoided.
- Can be stored for a longer period of times in a liquid nitrogen.

Disadvantages

- It needs controlled physiochemical environment (pH, temperature, osmotic pressure, O_2, CO_2, etc.)
- It is ten times more expensive for same quantity of animal tissue; therefore, reasons for its use should be compelling
- Unstable aneuploid chromosome (abnormal number of chromosome) constitution
- Controlled and defined **physiological conditions** (constitution of medium, etc.) are needed

Major differences *in vitro*

- Dissociation of cells from three-dimensional geometry of their counterpart *in vivo* stem propagate on a two-dimensional geometry substrate
- Specific cell interactions characteristic of the histology of the tissue are lost, when the cells spread out, it become mobile and in many cases, start to proliferate, so the growth fraction of the cell population increases
- When the cell lines forms, it will represent only one or two cell types, and many heterotypic cell-cell interaction are lost.
- Culture environment also lack the several systemic components involved in homeostatic regulation *in vivo.*
- Energy metabolism *in vitro* occurs largely by glycolysis, and although the citric acid cycle is still functional, it plays a lesser role.

TYPES OF TISSUE CULTURE

Three main methods of initiating a culture

1. ***Organ culture*** - the architecture characteristic of the tissue in vivo is retained, at least in part. The tissue is cultured at the liquid-gas interface favors the retention of a spherical or three-dimensional shape.

2. ***Primary explant culture -*** a fragment of tissue is placed at a glass (or plastic)-liquid interface, where, after attachment, migration is promoted in the plane of the solid substrate

3. ***Cell culture*** - the tissue, or outgrowth from the primary explant, is dispersed (mechanically or enzymatically) into a cell suspension, which may then be cultured as an adherent monolayer on a solid substrate or as a suspension in the culture medium

Concepts in mammalian cell culture

Most animals-**multicellular**

Cells specialization-perform specific functions

Can be induced to grow *in vitro* with special care (temp, pH, nutrients, etc)

Mononuclear cells can be released from soft tissues by enzymatic digestion (collagenase, trypsin, or pronase, which break down the ECM).

Alternatively, **explant culture** can be done –(pieces of tissue +appropriate substrate + rich nutrients medium following attachment, cell migration is promoted in the plane substrate

Classification of Cell Cultures

- **Primary Culture**
  - Cells taken directly from a tissue to a dish
  - Can be passages with a limited number of times. After the limit, the cell will die.

- **Culture of establish cell lines**
  - Established or immortal cell lines
  - Cells taken from a primary culture and passed or divided *in vitro.*
  - Can grow indefinitely in culture

Primary and Established Cell Lines

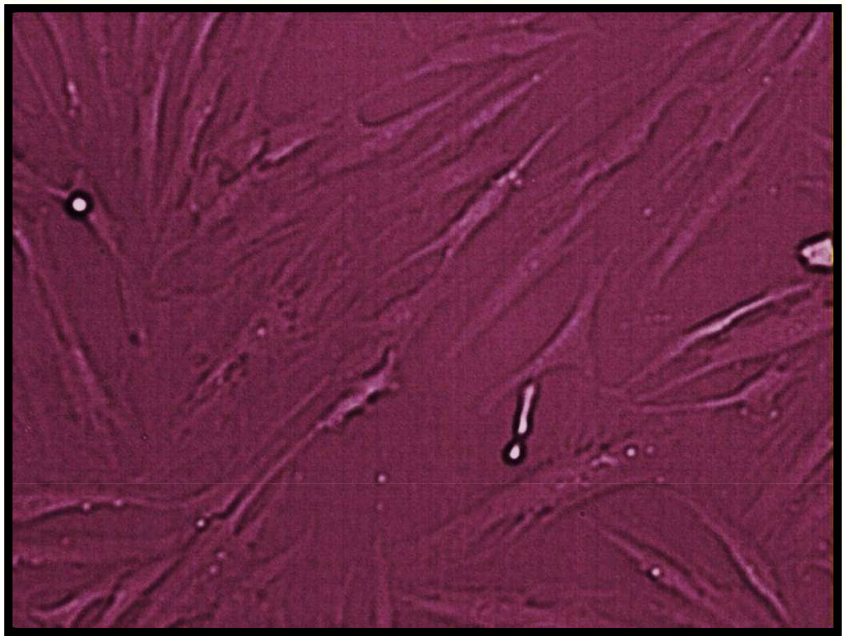
- A piece of tissue from the organism is usually quite complex and may contain, besides the specialized cells of the tissue, connective tissue cells, a variety of blood cells and reticutoendothelial cells (part of the immune system; consist of phagocytic cells).
- When a culture is first set up, these all survive for at least a day or two
- In usual culture condition many cells begin to migrate almost immediately
- The first cells to do this are often macrophages, followed by fibroblasts, which migrate in a radial manner from the explant
- Many of the specialized cells, however, remain immobile in the connective tissue stroma. Nerve cells, for example, usually remain in the explant although axons may migrate out from it.

- The subsequent fates of these cells vary enormously. Many of them are quite short-lived, e.g., most of the blood' cells die and disappear from the culture within two or three days.
- Other cells, such as neurons and muscle cells, survive in culture for months without dividing and then eventually die too.
- Still other cells begin to divide rapidly and continue to do so for sometime and many of these also die after a period, which varies from weeks to months.
- If the cells multiply repeatedly for a long time, they can often be 'passaged'.
- It is usually achieved by first obtaining the cells in suspension and then inoculating this into a new culture vessel along with fresh medium.
- As soon as cells have been **'passaged'** in this way the culture is known as primary cell line.

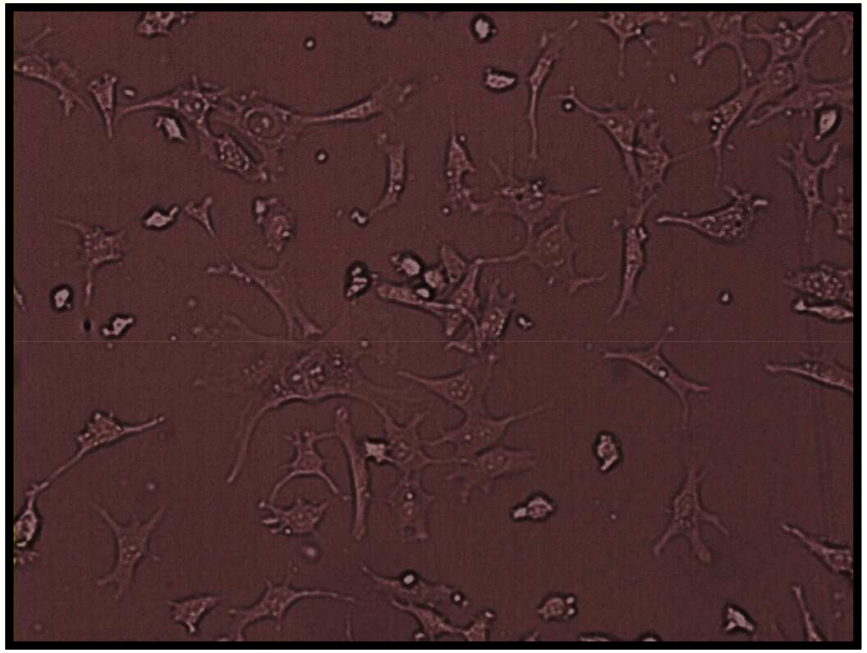
Primary and Established Cell Lines

- Frequently, primary cell lines go on dividing at quite a high rate for a long time and can be passaged repeatedly.
- Even after quite a large number of passage, some primary cell lines cease to proliferate and die out
- However, sometimes cell lines can be cultured for such a long time
- they apparently have developed the potential to be sub cultured indefinitely *in vitro*.
- Such cell lines are called established cell lines.
- As a general rule, a line is not designated as established cell line unless it has been sub cultured at least 70 times at intervals of 3 days between subcultures.

Fibroblast cells



Primary



Cell lines

- The formation of cell lines from a primary culture implies:
  - An increase in the total number of cells over several generation
  - The ultimate predominance of cells or cell lineage with the capacity for high growth, resulting in
  - A degree of uniformity in the cell population

- When cells are selected from the culture, by cloning or by some other method, the subline is known as a cell strain.

Primary and Established Cell Lines

Genetics of Cultured Cells

- Primary cell lines- **retain their diploid karyotype.**
- Transformed (established) cell lines - **variation in karyotype (aneuploid)**

Metabolism

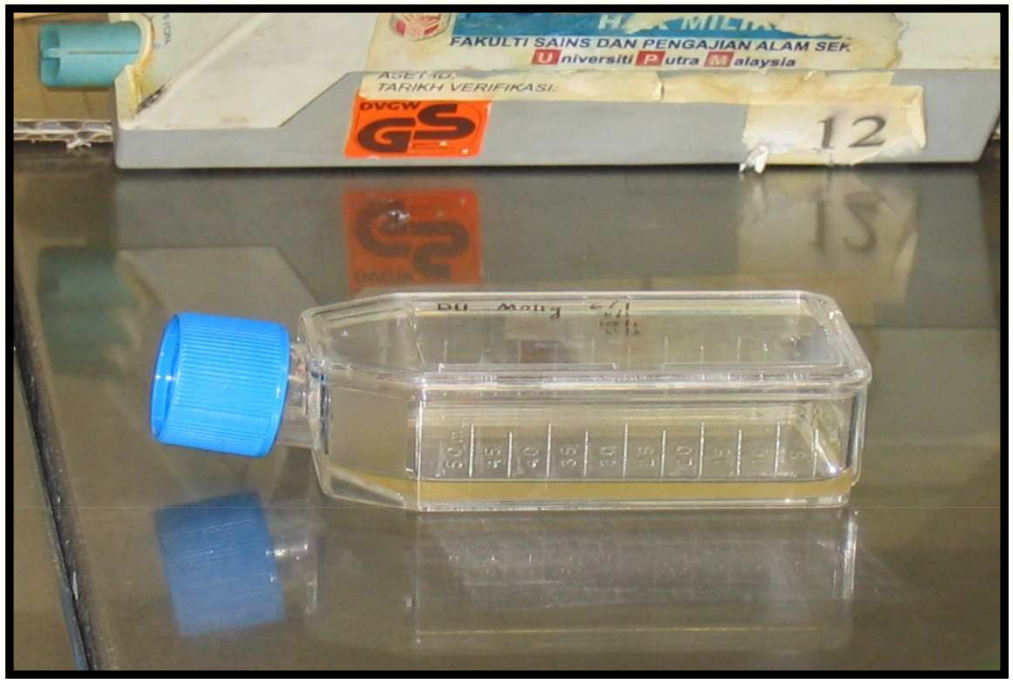
- In cultured cells, the metabolism of glucose proceeds by way of glycolytic and Krebs pathways as in the tissues of the intact organism.
- Cultured cells have very variable needs for oxygen.
- Cells can be grown in the complete absence of oxygen for rather short periods of time.
- Tissue culture cells can synthesize lipids and some cells are capable of synthesizing cholesterol.
- Cultured cells can build nucleic acids readily from simple compounds in a medium (such as formate, glycine and bicarbonate).
- They possess all the enzymes necessary for this purpose.

Primary cultures is used advantageously

1. The expense and inconvenience of maintaining an established cell stock is obviated (is bypassed or unnecessary).
2. They are particularly suitable for vaccine production since the probability of *in vitro* transformation of cells to malignancy is minimized.
3. Massive quantities of tissue are obtained conveniently for short-term studies.
4. They are hardy and can be sustained well in media of relatively simple composition.
5. Their degree and range of sensitivity to viral infection may exceed that of the established cell lines

The use of primary cell cultures has following inherent disadvantages:

1. The may be contaminated with latent viruses (e.g virus in monkey kidney tissues).
2. Long-term experiments concerning the biological properties of cells cannot be carried out.



Animal cell grow in a tissue culture flask