Assiut University

Workshop on

"The Basics for Culturing Animal Cells"

(18 – 20 Febraury 2008)

Under the auspices of

Prof. Dr. Ezzat A. Ahmed

President of Assiut University

Prof. Dr. Mohamed R. Bayoumi

Vice President of Assiut University for Higher Studies & Research

Prof. Dr. Asmaa A.A. Hussein

Director of the Molecualr Biology Unit

An introduction to cell and tissue cultures

By

Dr. Khaled Radad

Lecturer of Pathology

Faculty of Veterinary Medicine

Assiut University

2008

Tissue culture means:

The ability to **survive** and **grow** tissues outside the body in an artificial environment.

Embryo

Brain

Dissociated cells

Brief History:

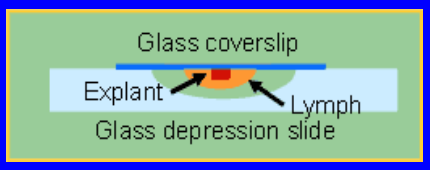
• In 1902, **Leo Loeb** placed a fragments of the skin from the embryos of guinea pigs in agar and in coagulated serum and inserted them into adult guinea pigs.

• He observed wandering and mitosis of the epithelial cells.

Pathologist, Leo Loeb 1869 - 1959

• In 1907, **Ross Harrison** discovered a way to grow cells outside the body.

• Harrison's first tissue culture:



Biologist, Ross Harrison
1870 - 1959

• At that time, "tissue culture" was a curiosity but in 1998, it was named as one of "medicine's ten greatest discoveries".

• **Alexis Carrel** and his colleagues are considered who actually built on Harrison's idea and laid the main principles for culturing tissues in an artificial media.

• They successfully solved three important problems that faced others before.

Surgeon, Alexis carrel
1873 - 1944

• These problems include **culture vessels**, **growing media** and **death** of cultured tissue.

1- Culture vessels:

In 1923, Carrel's lab developed the first practical cell culture flask (**D-Flask).**

Carrel's tissue culture flask

2- Growing media:

Montrose Burrows, an assistant to Carrel's, studied the formulation of the culture medium and replaced clotted lymph with plasma.

1- Death of cultured tissues:

- **Carrel** returned the cell death in the culture to the accunulation of waste products and exhuation of nutrients within the medium.
- He suggested that the tissue should removed from the culture substances that inhibit its growth to a new medium of development.
- Moreover, they subcultured tumor explants and

developed the first cell lines which were kept growing for up to several months.

• Tissue culture techniques developed and refined in Carrel's laboratory had become the methods used for most cell culture reserch in laboratories around the world and,

• Very little changed in culture technology till the year, 1950s.

Since 1950, tissue culture technology has been greatly developed due to many factors:

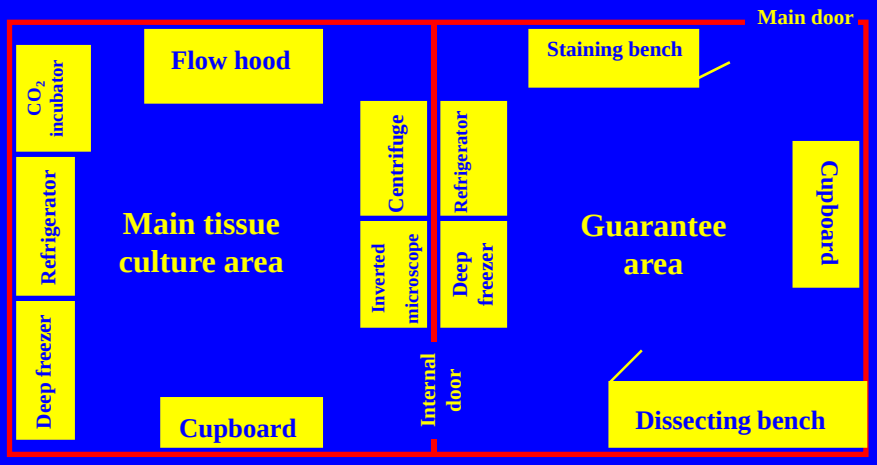
• The need for production of antiviral vaccines and antibodies and understanding of neoplasia.

• The technical improvements made by commercial supply of media and sera, control of contamination with antibiotics and the use of clean-air equipment.

• Pressure made by animals' rights groups over the unnecessary use of experimental animals.

General requirements for culturing tissues:

I- Tissue culture laboratory:



II- Equipment:

1- Laminar flow hood:

It provides clean air to the working area which:

- Suspends and removes contaminants introduced during work.
- Prevents room air from entering the hood.

Laminar flow hood

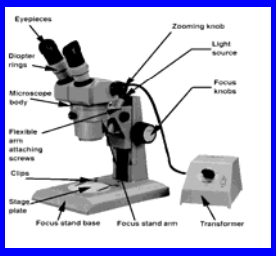
2- Incubator:

- Temperature (28 - 37 °C).
- Humidity (100%).
- CO_2 level (5-10%).

Incubator

3- Dissecting microscope:

Used for dissecting and obtaining target tissues in case of primary cultures.



Dissecting microscope

4- Inverted microscope:

Used for observing the growth status of cultured tissues.

Inverted microscope

5- Inverted fluorescence microscope:

Based on the phenomena that certain material emits energy detectable as visible light when irradiated with the light of specific wavelength.

Fluorescence microscope

Hot air oven

Autoclave

Water bath

Refrigerator

Deep freezer

Water distiller

Syringe filters

Vacuum pump

Magnetic stir plate

Syringe filters

Electric pipette

pH meter

Mechanical pipette

Types of tissue cultures:

- **Primary tissue culture.**
- **Cell lines.**

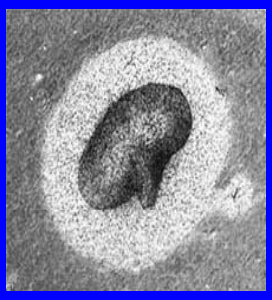
I- Primary tissue culture:

Refers to cultures prepared from tissues taken directly from animals.

It includes:

1- Organ culture:

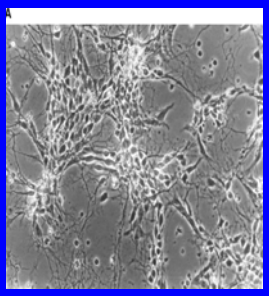
Means the maintenance of a piece of tissue, a part of organ or a whole organ in vitro.



Organ culture

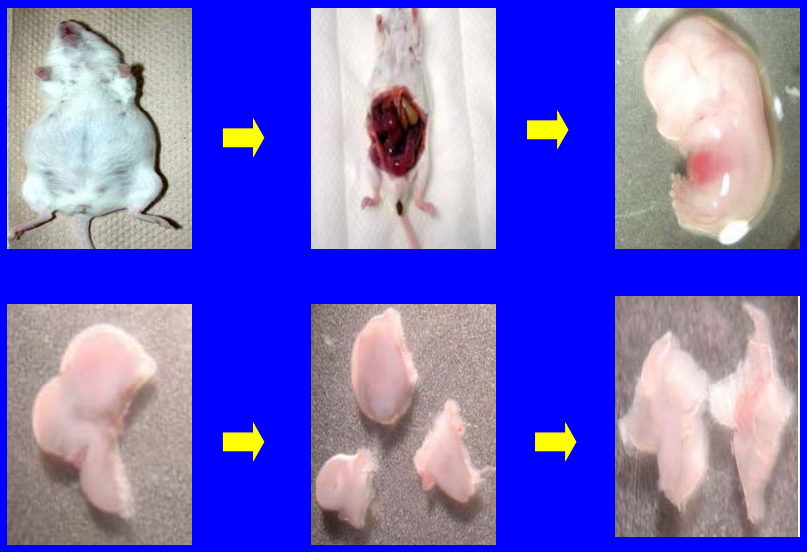
2- Primary cell culture:

Obtained when taken tissue is dissociated, mechanically or enzymatically, into single cells which could be plated on a coated surface.

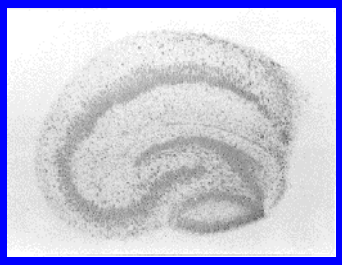


Primary cell culture

For example, primary mesencephalic cell culture:



3- Slice tissue culture:



Hippocampus slice tissue culture

• Cultures developed primarily by **Harrison** would now be referred to as explant or organotypic cultures.

• In which, small pieces of tissue of interest are simply allowed to attach to an appropriate substrate and are cultured in enriched media.

4- Reaggregate culture:

• Dissociated cells is kept in suspension rather than allowed to settle on and attach to solid substrate.

• In which, cells tend to reaggregate into small balls.

• This type of culture permit cells to develop in three dimensions.

5- Histotypic or histoculture:

High density slice culture of one cell type.

II- Cell line:

• Primary cell cultures can be passaged a finite number of passages before reaching a crisis.

• Passages before crisis are referred to as a **cell strains**.

B16-F10 Melanoma Cell Line

• Cells that survive the crisis and continue to grow are referred to as a **cell line**.

Types:

1- Continuous cell line:

Population of cells that can be passaged indefinitely and express reasonable stable phenotype.

2- Transformed cell line:

Cell lines obtained from tumor cells.

3- Clonal cell line:

Cells could be cloned in continuous cell lines to obtain genetically homogenous population.

Thank you for your interest