Assiut University

Workshop on
"The Basics for Culturing Animal Cells"
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An introduction to cell and tissue cultures

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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 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:

![Diagram of tissue culture](https://via.placeholder.com/150)

• 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.

• 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).

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 accumulation of waste products and exhaustion of nutrients within the medium.
- He suggested that the tissue should be 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 research 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:

- Flow hood
- Staining bench
- Centrifuge
- Refrigerator
- Deep freezer
- Cupboard
- Internal door
- Inverted microscope
- Main door
- Guarantee area
- Main tissue culture area
- Dissecting bench
- CO₂ incubator
- Cupboard
- Main door

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.
2- **Incubator:**
• Temperature (28 - 37 °C).
• Humidity (100%).
• CO₂ level (5-10%).

3- **Dissecting microscope:**
Used for dissecting and obtaining target tissues in case of primary cultures.

4- **Inverted microscope:**
Used for observing the growth status of cultured tissues.

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.
Hot air oven
Autoclave
Water bath
Refrigerator
Deep freezer
Water distiller
Vacuum pump
Magnetic stir plate
Syringe filters
Electric pipette
pH meter
Mechanical pipette
Types of tissue cultures:

• Primary tissue culture.
• Cell lines.

1- 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.

2- Primary cell culture:

Obtained when taken tissue is dissociated, mechanically or enzymatically, into single cells which could be plated on a coated surface.
For example, primary mesencephalic cell culture:

3- 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**.
- 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