University of Basrah

College of Science

Department of Pathological Analysis

Course title: Tissue Culture Techniques

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By

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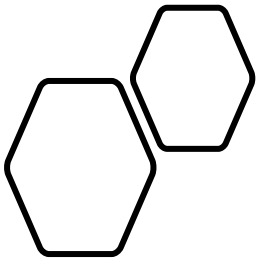
1- Course description:



This module is a basic requirement for the department. It provides insights into the application of animal tissue culture .It seeks to familiarize students to the basic principles of tissue culture, and to expose them to its many applications, the theory production of transgenic tissue will be discussed. The course involves correlation with practical course to ensure full understanding of the techniques, the lab will provide hands experience in many of the methods discussed.

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2- Course objectives: -

Describe the Equipment's used in animal tissue culture. - Understand the safety procedures need for tissue culture. - Understand techniques used in tissue culture.

3- Books: -

Culture of Animal Cells, A manual of basic technique, 5th Edi on by Freshney, RI. WIELY-LISS,2004(optional) –

Experiments in animal tissue culture, 3ed edi on by J.H.Dodds and L.W.Roberts. Cambridge university press,1999,(optional)

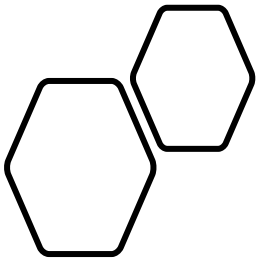
Animal Tissue Culture

-Introduction

-Historical Background

What is Cell Culture?

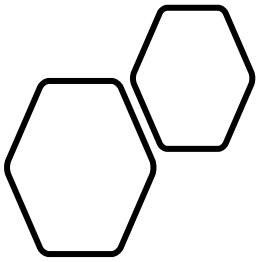
Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been already established.



In other words

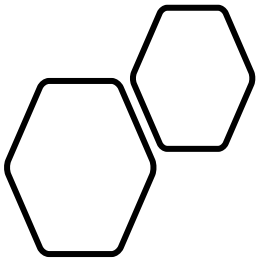
In modern usage, **tissue culture** generally refers to the growth of cells from a tissue from a multicellular organism *in vitro*. These cells may be cells isolated from a donor organism, "primary cells", or an immortalised cell line. The cells are bathed in a culture medium, which contains essential nutrients and energy sources necessary for the cells' survival.[The term tissue culture is often used interchangeably with **cell culture**

Primary Culture



Primary culture refers to the stage of the culture after the cells are isolated from the tissue and proliferated under the appropriate conditions until they occupy all of the available substrate (i.e., reach confluence). At this stage, the cells have to be subcultured (i.e., passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth.

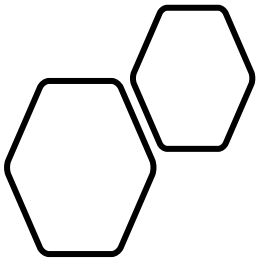
Cell Line



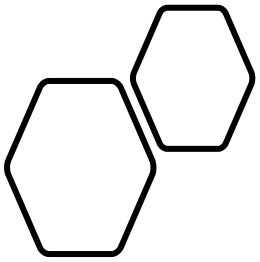
After the first subculture, the primary culture becomes known as a cell line or subclone. Cell lines derived from primary cultures have a limited life span (i.e., they are finite), and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population.

Cell Strain

If a subpopulation of a cell line is positively selected from the culture by cloning or some other method, this cell line becomes a cell strain. A cell strain often acquires additional genetic changes subsequent to the initiation of the parent line



Finite vs Continuous Cell Line

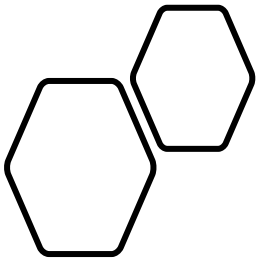


Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as senescence; these **cell lines are known as finite**.

However

Some cell lines become immortal through a process called transformation, which can occur spontaneously or can be chemically or virally induced. When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a **continuous cell line**.

Culture Conditions



Culture conditions vary widely for each cell type, but the artificial environment in which the cells are cultured invariably consists of a suitable vessel containing a substrate or medium that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals), growth factors, hormones, and gases (O2, CO2), and regulates the physicochemical environment (pH, osmotic pressure, temperature). Most cells are anchoragedependent and must be cultured while attached to a solid or semi-solid substrate (adherent or monolayer culture), while others can be grown floating in the culture medium (suspension culture).

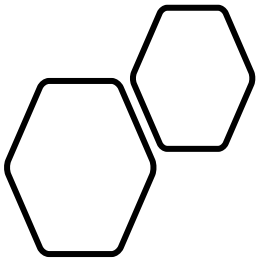
Cryopreservation

If a surplus of cells are available from subculturing, they should be treated with the appropriate protective agent (e.g., DMSO or glycerol) and stored at temperatures below –130°C (cryopreservation) until they are needed.

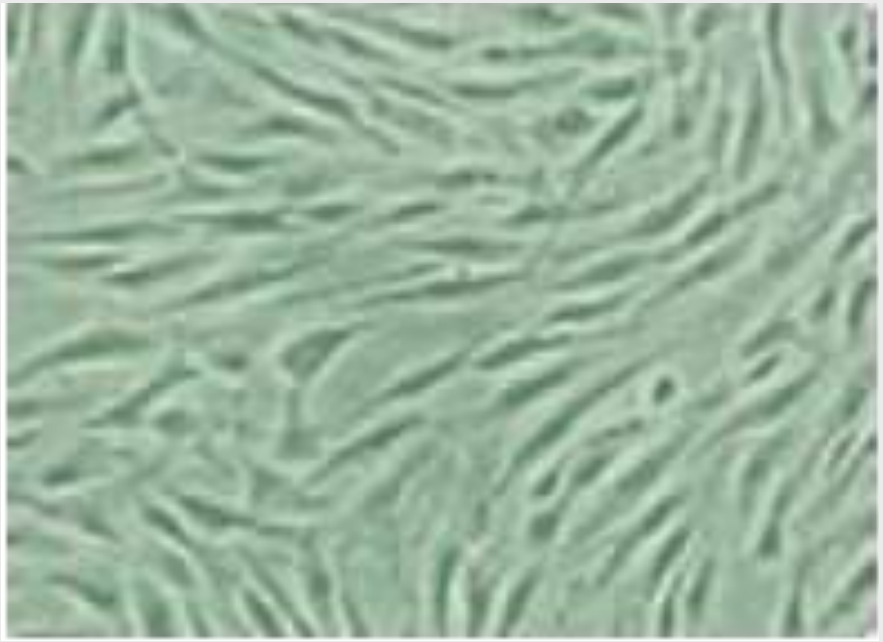
For more information on subculturing and cryopreserving cells, refer to the Guidelines for Maintaining Cultured Cells

Morphology of Cells in Culture

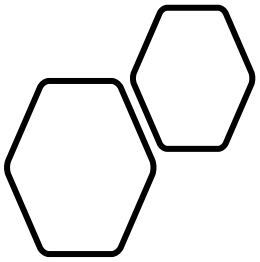
Cells in culture can be divided in to three basic categories based on their shape and appearance (i.e., morphology).

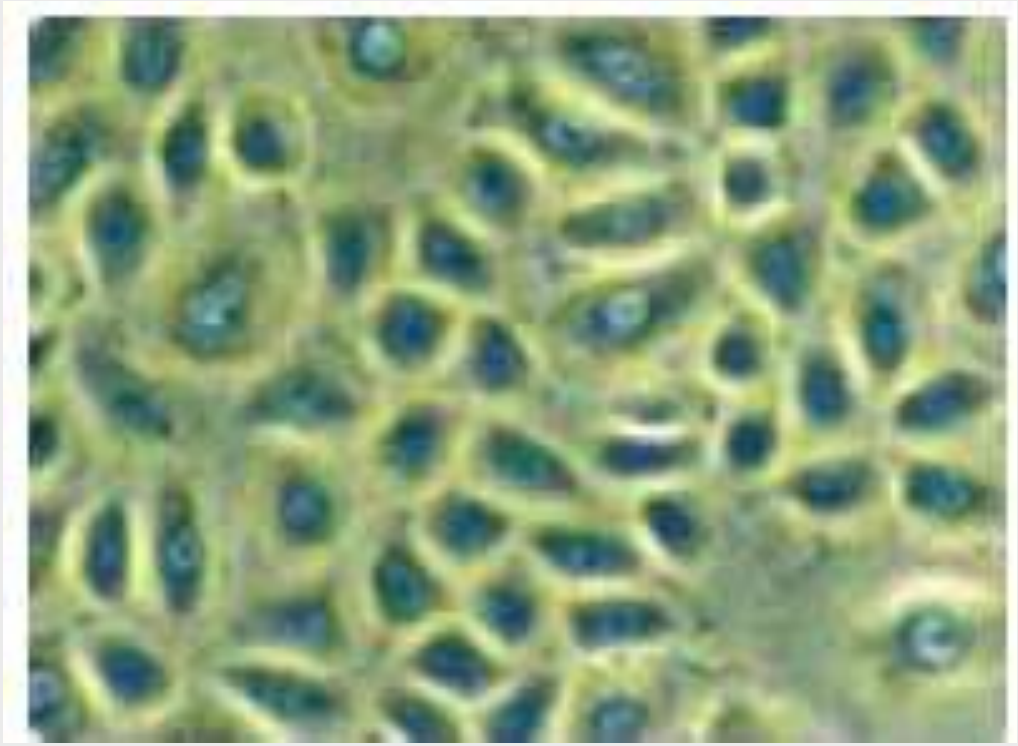


Fibroblastic (or fibroblast-like) cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate.

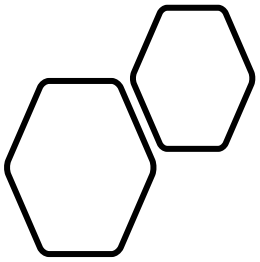


Epithelial-like cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.





Lymphoblast-like cells are spherical in shape and usually grown in suspension without attaching to a surface.



Applications of Cell Culture

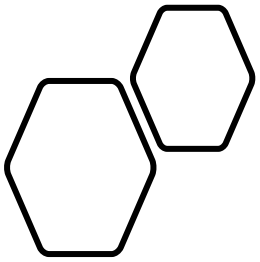
Cell culture is one of the major tools used in

-**cellular and molecular biology**, providing excellent model systems for studying the normal physiology and biochemistry of cells (e.g., metabolic studies, aging)

-**the effects of drugs and toxic compounds on the cells**, and mutagenesis and carcinogenesis. It is also used in drug screening and development, and large scale manufacturing of biological compounds (e.g., vaccines, therapeutic proteins).

-The major advantage of using cell culture for any of the these applications is the consistency and reproducibility of results that can be obtained from using a batch of clonal cells.

Historical Background



- **1878: Claude Bernard** , proposed that physiological systems of an organism can be maintined in a living system after the death of an organism.

1885: Roux maintained embryonic chick cells in saline culture. -

- **1897: Loeb** demonstrated the survival of cells isolated from blood and connective tissue in serum and plasma. -

- **1907: Harrison:** cultivated frog nerve cells in a lymp clot and obsrerved the grouth of nerve fibers in vitro for several weeks.He was considered by some as the father of cell culture. -

1910: Burrows succeeded in long term cultivation of chicken embryo cell plasma clots. He made detailed observation of mitosis -

1911: Lewis and Lewis, Made the first liquid media consisted of sea water, serum, embryo extract, salts and peptones.

1913: Carrel introduced strict aseptic techniques so that cells could be cultured for long periods.

1916: Rous and Jones introduced photolytic enzyme trypsin for the subculture of adherent cells.

1940s: the use of the antibiotics penicillin & streptomycin in culture medium decreased problems of contamination in cell culture.

1948: Earle , isolated mouse L fibroblasts which formed clones from single cells.

1952: Gey established a continuous cell line from a human cervical carcinoma known as HeLa (Helen Lane) cells . https://en.wikipedia.org/wiki/Henrietta_Lacks .

1952: Dulbecco developed plaque assay for animal viruses using confluent monolayers of cultured cells.

1955: Eagle Studied the nutrient requirement of selected cells in culture & first widely used chemically defined medium

1965: Harris and Watkins were able to fuse human and mice cells by use of a virus.

1975: Kohler and Milstein produced the first hybridoma capable of secreting a monoclonal antibody.

1978: Sato established the basis for the development of serum-free media from cocktails of hormones and growth factors.

1982: Human insulin become the first recombinant protein to be licensed as a therapeutic agent.

1985: Human growth hormone produced from recombinant bacteria was accepted for therapeutic use.

Major development in tissue culture technique

First development was use of antibiotic which inhibits the growth of contamination.

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.

Thank you