Tissue culture

Tissue culture as a technique was first used almost 100 years ago to elucidate some of the most basic questions in developmental biology.

Ross Harrison at the Rockefeller Institute, in an attempt to observe living, developing nerve fibers, cultured frog embryo tissues in plasma clots for 1 to 4 weeks (Harrison, 1907). He was able to observe the development and outgrowth of nerve fibers in these cultures. In 1912, Alexis Carrel, also at the Rockefeller Institute, attempted to improve the state of the art of animal cell culture with experiments on the culture of chick embryo tissue.

The next important advance in the conceptualization and technology of cell culture was the demonstration by Katherine Sanford and co-workers (1948) that single cells could be grown in culture. This, along with Harry Eagle's (1955) demonstration that the complex tissue extracts, clots, and so forth previously used to grow cells could be replaced by " . . . an arbitrary mixture of amino acids, vitamins, co-factors, carbohydrates, and salts, supplemented with a small amount of serum protein . . . " opened up a new area of cell culture.

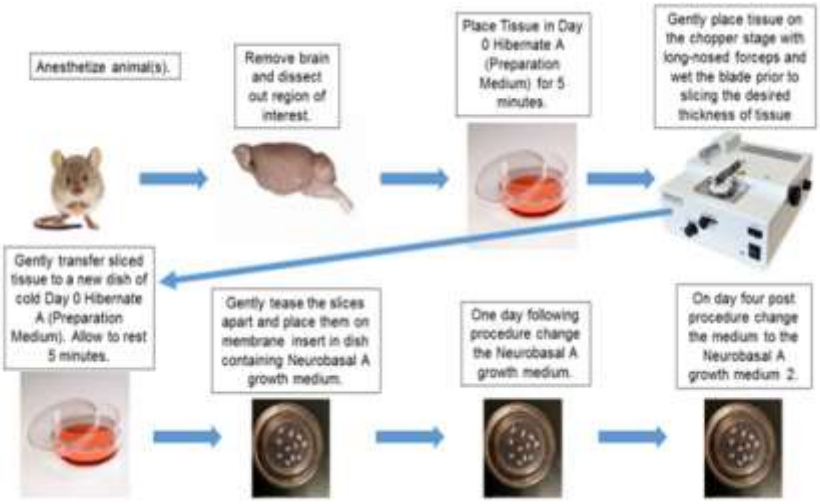
Defination - it is the activity to Faciliting the growth of tissue or cells in an artificial medium separate from the organism.

This is typically facilitated via use of liquid,semi-solid or solid growth medium such as broth or agar.

The term tissue culture is coined by American pathologist **Montrose Thomas burrows.**

The Various types of tissue culture :-

1. **Organ culture :** slice of organ can be maintained in vitro for days weeks so that it mimics the actual morphology of the tissue and it's function . Organ culture is useful for viruses which are highly specialized parasites of certain organs Ex:- Tracheal ring organ culture is used for the isolation of coronavirus, a respiratory pathogen.



2. **Exlant culture:** Frag ments of minced(very small pieces)can be grown as explants embedded in plasma clots. They may also be cultivated in suspension. Adenoid tissue explants culture were used for the isolation of adenovirus.

3. **Cell culture:** It is widely used for growing viruses. Tissues are dissociated into the component cells by the action of enzyme and mechanical shaking . The cells are washed ,counted and suspended in a growth medium.The growth medium conists of essential amino acids,glucose,vitamins , salts and a buffer.Antibiotics are added to prevent bacterial contamination . the cell suspension is put into bottles , tubes and pertidishes.
The cell adhere to the glass or plastics surface , divide and form a confluent monplayer sheet within a week . Cell culture is further classified on the basis of origin,chromosomal characters and the number of generations through which they can be maintained . It is of three types- primary cell culture,diploid cell strain and continuous cell lines.

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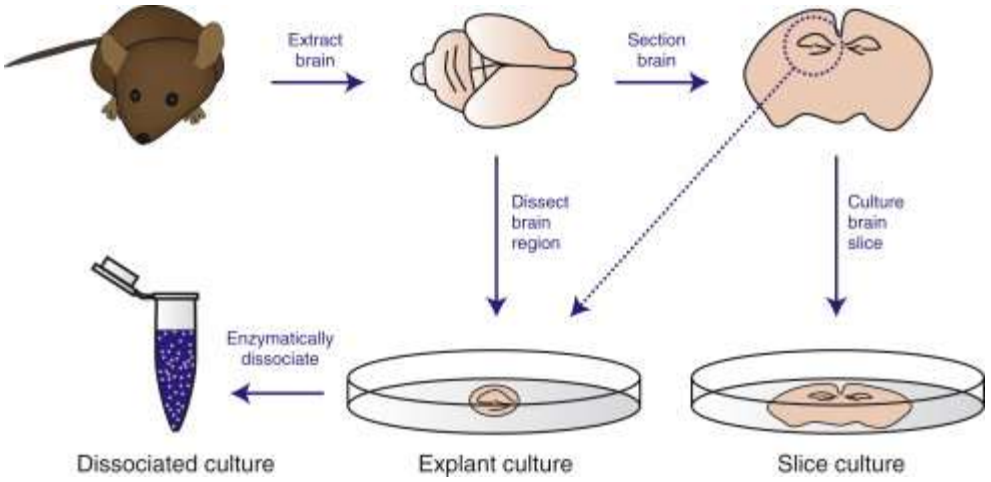
- **Primary cell culture**
- **Diploid cell strain**
- **Continuous cell culture**

Primary cell culture are normal cells freshly taken from the body and cultured. They are capable of only limited growth in culture. They cannot be maintained in serial culture example :- monkey kidney, human embryo kidnry and chick embryo cell culture .

Primary Cultures Primary cultures are derived directly from excised, normal animal tissue and cultures either as an explant culture or following dissociation into a single cell suspension by enzyme digestion.

Such cultures are initially heterogeneous but later become dominated by fibroblasts. The preparation of primary cultures is labour intensive and they can be maintained in vitro only for a limited period of time.

During their relatively limited lifespan primary cells usually retain many of the differentiated characteristics of the cell in vivo. Important Note: Primary cultures by definition have not been passaged, as soon as they are passaged they become a cell line and are no longer primary. 'Primary' cells sourced from most suppliers are in fact low-passage cell lines.



Diploid cell strains are cells of a single type that retain the original diploid chromosome number and karyotype during serial subcultivations for a limited number of times .After about fifteen serial passages, they undergo 'senescence'. Diploid strain developed from human fibroblast are a good example .

Continuous cell lines are cells of a single type , usually derived from cancer cells . they are capable of continuous serial cultivation indefinitely .

Hela cell are derived from carcinoma of cervix. Cell culture is used for the isolation of viruses an their cultivation for vaccines production.

Continuous cultures are comprised of a single cell type that can be serially propagated in culture either for a limited number of cell divisions (approximately thirty) or otherwise indefinitely.

Cell lines of a finite life are usually diploid and maintain some degree of differentiation. The fact that such cell lines senesce after approximately thirty cycles of division means it is essential to establish a system of Master and Working banks in order to maintain such lines for long periods. Continuous cell lines that can be propagated indefinitely generally have this ability because they have been transformed into tumour cells.

Tumour cell lines are often derived from actual clinical tumours, but transformation may also be induced using viral oncogenes or by chemical treatments.
Transformed cell lines present the advantage of almost limitless availability, but the disadvantage of having retained very little of the original in vivo characteristics.

Application-

1. Microbial detection in mammalin cell culture system.
2. Facilited progress in therapeutics and regenerative medicines.
3. Cell lines are used as model for drug studies.
4. Drug screening in cancer cell lines.
5. Cell culture model for drug Permeability studies.

The Process To Culture Cells

Harvest Cells

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Isolate Cells with the use of appropriate enzymes

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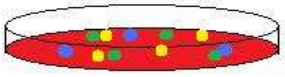
Apply the isolated cells on to an appropriate growth media in a culture dish



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Culture cells by placing the culture dish in a cell incubator

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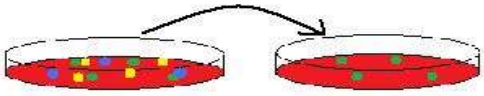
Subculture cells to obtain a pure culture or to bypass some problems (such as senescence)



Verify the cultured cells are of the cell type of interest

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Cells are ready to be manipulated or modified for experimental procedures

The cell lines most commonly ordered from ECACC are listed

Table 1. Commonly used cell lines of each culture type

Attached Cell Lines		
Name	**Species and tissue of origin**	**Morphology**
MRC-5	Human lung	Fibroblast
HeLa	Human cervix	Epithelial
Vero	African Green Monkey Kidney	Epithelial
NIH 3T3	Mouse embryo	Fibroblast
L929	Mouse connective tissue	Fibroblast
CHO	Chinese Hamster Ovary	Fibroblast
BHK-21	Syrian Hamster Kidney	Fibroblast
HEK 293	Human Kidney	Epithelial
Hep G2	Human Liver	Epithelial
BAE-1	Bovine aorta	Endothelial
SH-SYSY	Human neuroblastoma	Neuroblast
Suspension Cell Lines		
Name	**Species and tissue of origin**	**Morphology**
NS0	Mouse myeloma	Lymphoblastoid
U937	Human Hystiocytic Lymphoma	Lymphoblastoid
Namalwa	Human Lymphoma	Lymphoblastoid
HL60	Human Leukaemia	Lymphoblastoid
WEHI 231	Mouse B-cell Lymphoma	Lymphoblastoid
YAC 1	Mouse Lymphoma	Lymphoblastoid
U 266B1	Human Myeloma	Lymphoblastoid