University of Baghdad college of Medicine
2023- 2024

Cell Lines

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Introduction to Cell lines

- A cell line is a **permanently established cell culture** that will proliferate indefinitely given appropriate fresh medium and space.

- A cell culture developed from a single cell and therefore consisting of cells with a uniform genetic make-up.

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 established.

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 or **subclone**

- After the **first** subculture, the primary culture becomes known as a **cell line** or **subclone**.
- The term cell line refers to the **propagation of culture after the first subculture**
- 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.

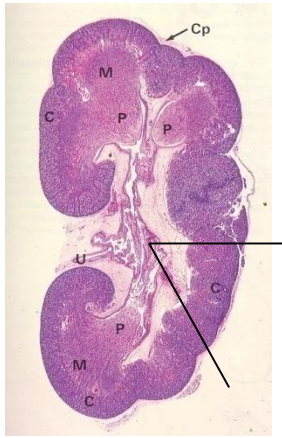
Cell strain

- 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.

Cell culture

- Mouse, mammals,
- Embryo
- Embryonated Eggs

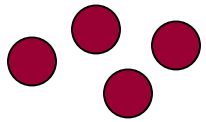
because stage of differentiation)



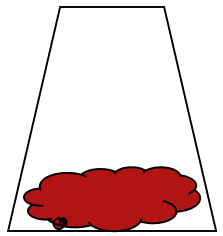
explant

organ

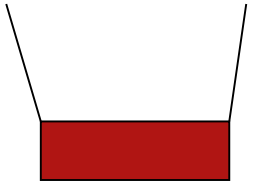
Finely cut



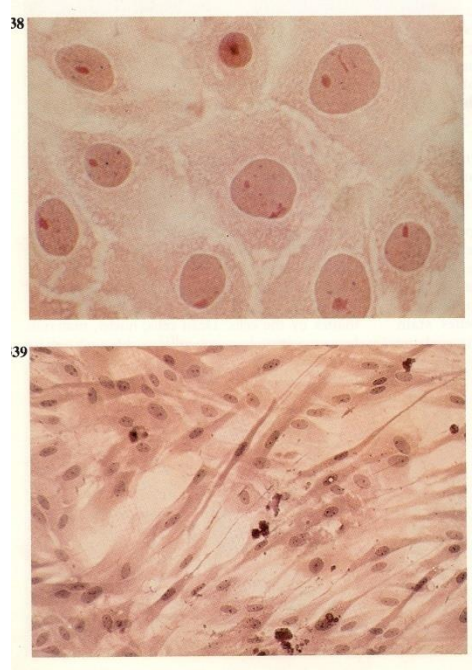
Finely cut
tissue or explant



Enzymic digestion

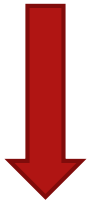


Grow in media
-monolayer
-suspension cells



STAGES OF CULTURE

Isolated tissue



(disaggregation)

Primary cell culture(limited lifespan after certain proliferations undergo senescence)

Finite cell cultures

continuous cell lines(immortalized cell line acquires ability to proliferate indefinitely by transformation)

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 the following:

- a **substrate or medium** that supplies the essential nutrients (amino acids, carbohydrates, vitamins, minerals)
- **growth factors**
- **hormones**

- **gases (O_2, CO_2)**
- a regulated **physico-chemical environment** (pH, osmotic pressure, temperature)
- Most cells are **anchorage-dependent** 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**).

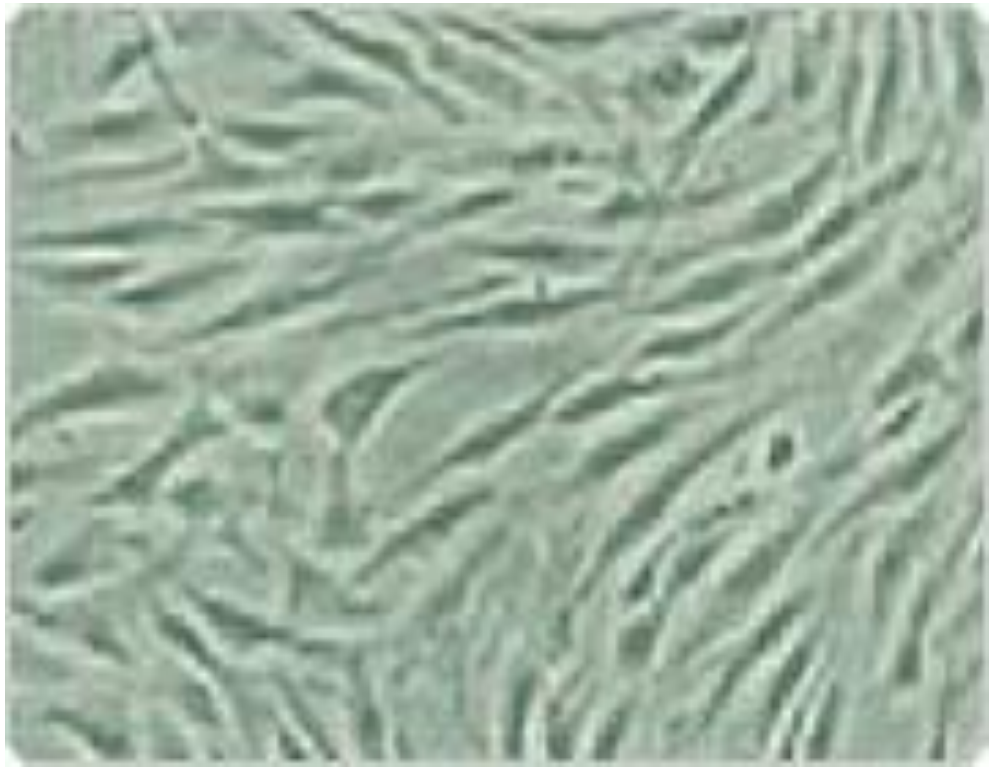
Cryopreservation

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.

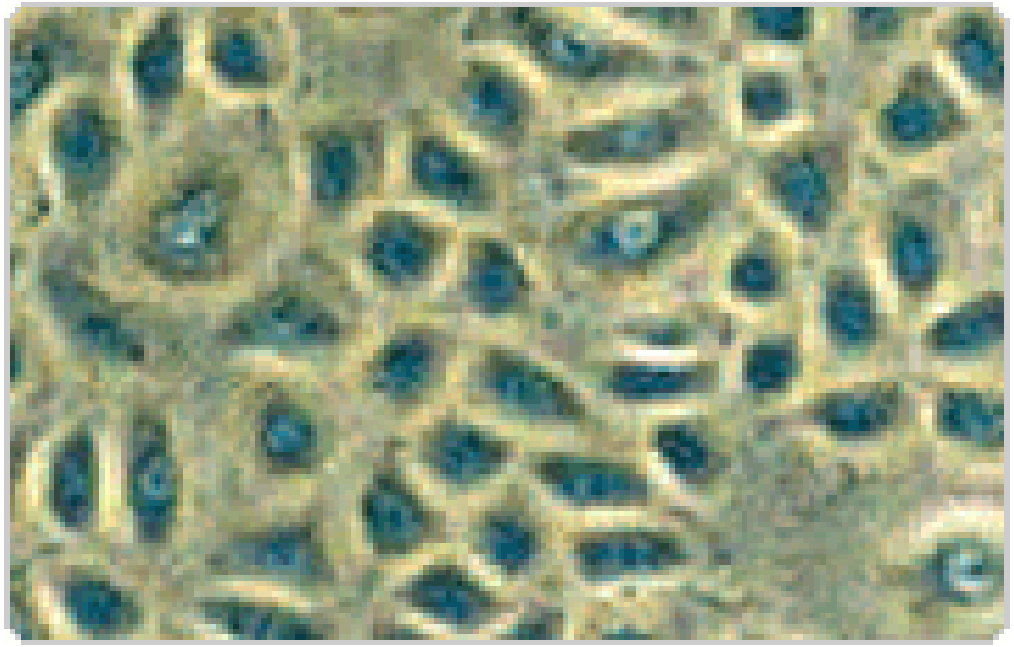
Morphology of Cells in Culture

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

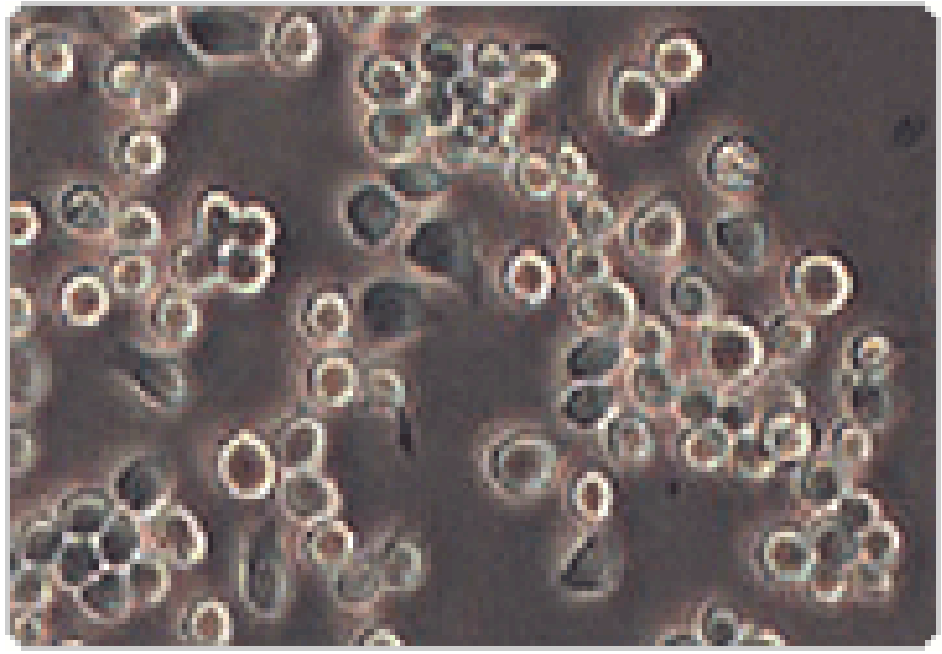
Fibroblastic (or fibroblast-like) cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate.



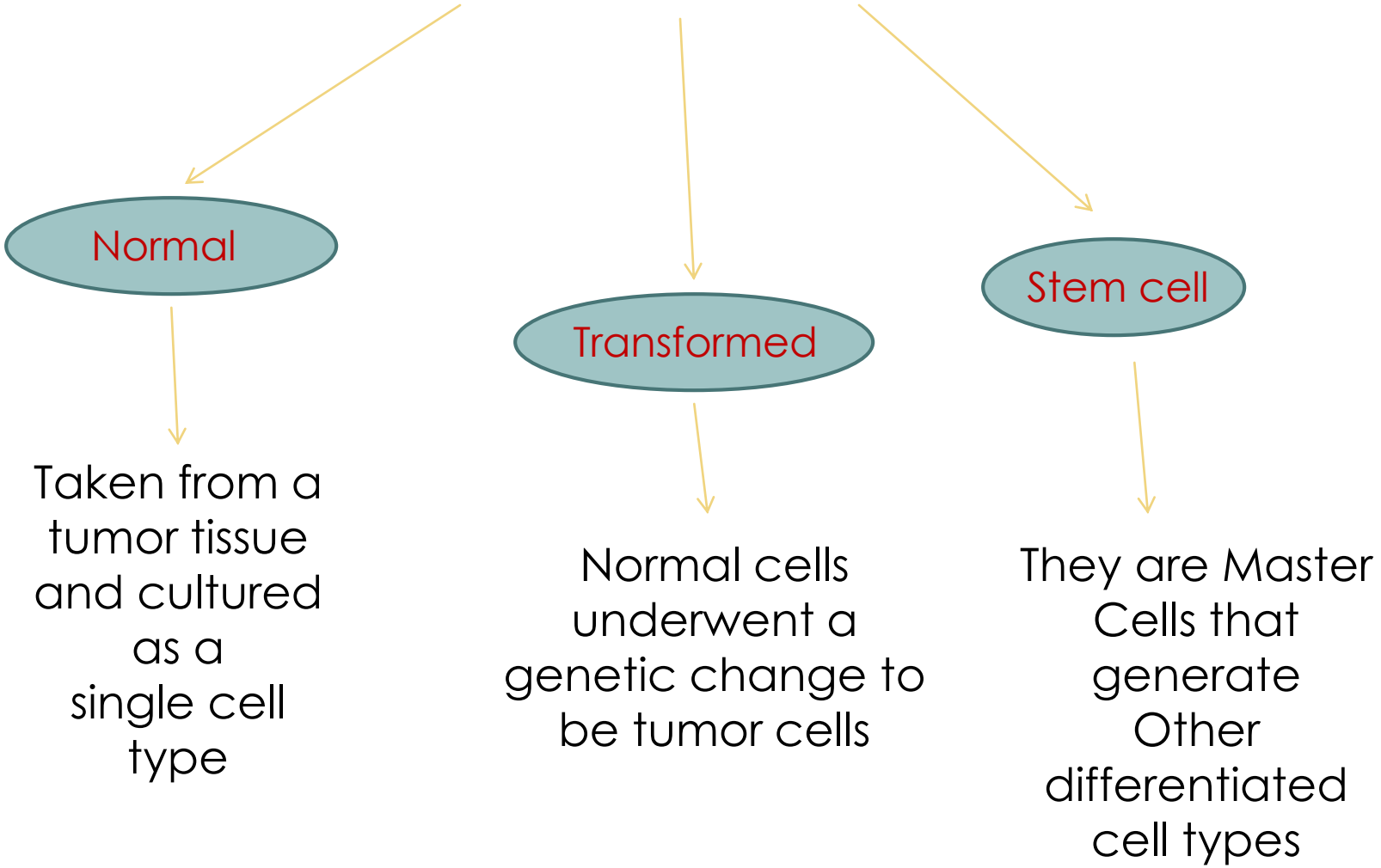
Epithelial-like cells are polygonal in shape with more regular dimensions, and grow attached to a substrate in discrete patches.



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



Cell line Types



Types of Cell Lines:

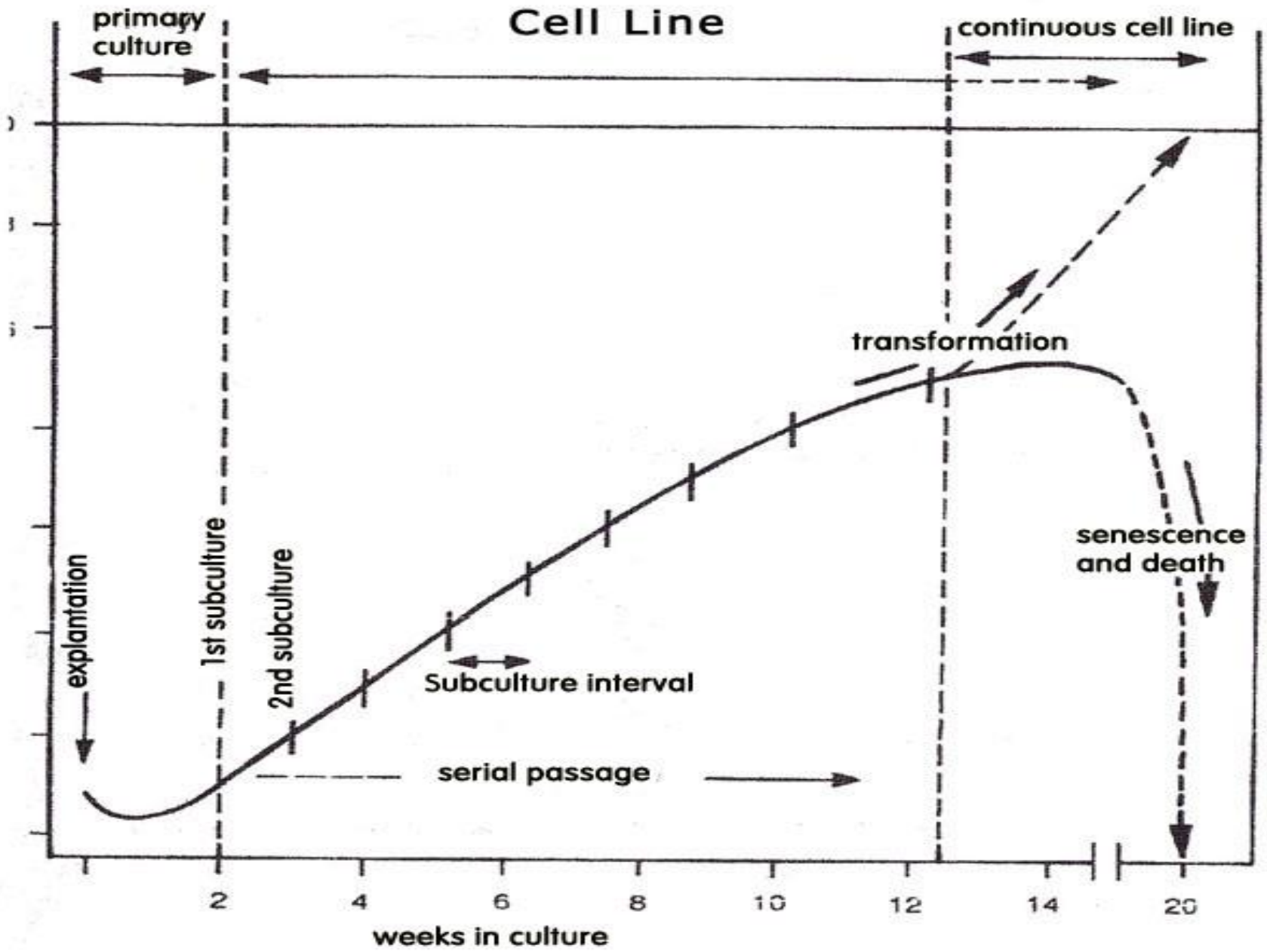
- Finite cell lines
- Continuous cell lines

Finite Cell Lines :

- 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**.
- The cells normally divide 20 to 100 times (i.e. is 20-100 population doublings) before extinction. The actual number of doublings depends on the species, cell lineage differences, culture conditions etc.
- The human cells generally divide 50-100 times, while murine cells divide 30-50 times before dying.

Continuous Cell Lines or Immortal cell lines

- When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a **continuous cell line**.
- The continuous cell lines are **transformed, immortal and tumorigenic**. The transformed cells for continuous cell lines may be obtained from normal primary cell cultures (or cells strains) by treating them with chemical **carcinogens or by infecting with oncogenic viruses.**


Cell Line
primary culture
continuous cell line
transformation
senescence and death
explantation
1st subculture
2nd subculture
Subculture interval
serial passage
2
4
6
8
10
12
14
20
weeks in culture

Property	Finite cell line	Continuous cell line
➢ Growth rate	Slow	Fast
➢ Mode of growth	Monolayer	Suspension or Monolayer
➢ Yield	Low	High
➢ Transformation	Normal	Immortal, tumorigenic
➢ Ploidy	Euploidy	Aneuploid
➢ Anchorage dependence	Yes	No
➢ Contact inhibition	Yes	No
➢ Cloning efficiency	Low	High
➢ Serum requirement	High	Low
➢ Markers	Tissue specific	Chromosomal, antigenic or enzymatic

Comparison of properties of finite and continuous cell lines

Nomenclature of Cell Lines:

- It is a common practice to give codes or designations to cell lines for their identification. For instance, the code **NHB 2-1** represents the cell line from normal human brain, followed by cell strain (or cell line number) 2 and clone number 1.
- While naming the cell lines, it is absolutely necessary to ensure that each **cell line designation is unique** so that there occurs no confusion when reports are given in literature.
- Further, at the time of publication, the-cell line should be prefixed with a code designating the **laboratory from** which it was obtained e.g. NCI for National Cancer Institute, WI for Wistar Institute.

Selecting the Appropriate Cell Line

Consider the following criteria for selecting the appropriate cell line for your experiments:

Species:

In general, **non-human cell lines** have less risk of biohazards, hence preferred. However, species differences need to be taken into account while extrapolating the data to humans.

Functional characteristics: What is the purpose of your experiments? For example, liver- and kidney-derived cell lines may be more suitable for toxicity testing.

Finite or continuous cell lines:

Cultures with continuous cell lines are preferred as they grow faster, easy to clone and maintain, and produce higher yield. But it is doubtful whether the continuous cell lines express the right and appropriate functions of the cells. Therefore, some workers suggest the use of finite cell lines, although it is difficult.

Normal or transformed:

Transformed cell lines usually have an increased growth rate and higher plating efficiency, are continuous, and require less serum in media, but they have undergone a permanent change in their phenotype through a genetic transformation.

Growth characteristics:

The following growth parameters need to be considered:

i. Population doubling time

ii. Ability to grow in suspension

iii. Saturation density (yield per flask)

iv. Cloning efficiency

Stability:

The stability of cell line with particular reference to cloning, generation of adequate stock and storage are important.

Phenotypic expression:

It is important that the cell lines possess cells with the right phenotypic expression.

EXAMPLES OF ESTABLISHED CELL LINES

- May be derived from Normal or Tumor cells.

Cell line	Organism	Origin Tissue
HeLa	Human	Cervical cancer
293-T	Human	Kidney (embryonic)
A-549	Human	Lung carcinoma
ALC	Murine	Bone marrow
CHO	Hamster	Ovary
HB54	Hybridoma	Hybridoma
FM3	Human	Metastatic lymph node

Animal cell lines and products

Cell line	Product
Human tumour	Angiogenic factor
Human leucocytes	Interferon
Mouse fibroblasts	Interferon
Human Kidney	Urokinase
Transformed human kidney cell line, TCL-598	Single chain urokinase-type plasminogen activator (scu-PA)
Human kidney cell (293)	Human protein (HPC)
Dog kidney	Canine distemper vaccine
Cow kidney	Foot and Mouth disease (FMD) vaccine
Chick embryo fluid	Vaccines for influenza, measles and mumps
Duck embryo fluid	Vaccines for rabies and rubella
Chinese hamster ovary (CHO) cells	Tissue-type plasminogen activator (t-PA) B-and gamma interferons Factor VIII

Applications

- Screening of the anti cancer drugs
- Cell based bioassay
- To determine the cytotoxicity
- *In vitro* screening of several drugs
- Production of antiviral vaccines
- Cancer research, which required the study of uncontrolled cell division in cultures
- Cell fusion techniques
- Genetic manipulation
- Study of the effects of toxins & pollutants using cell lines
- Study of the function of nerve cells
- Chromosome analysis of cells derived from womb

Thank you