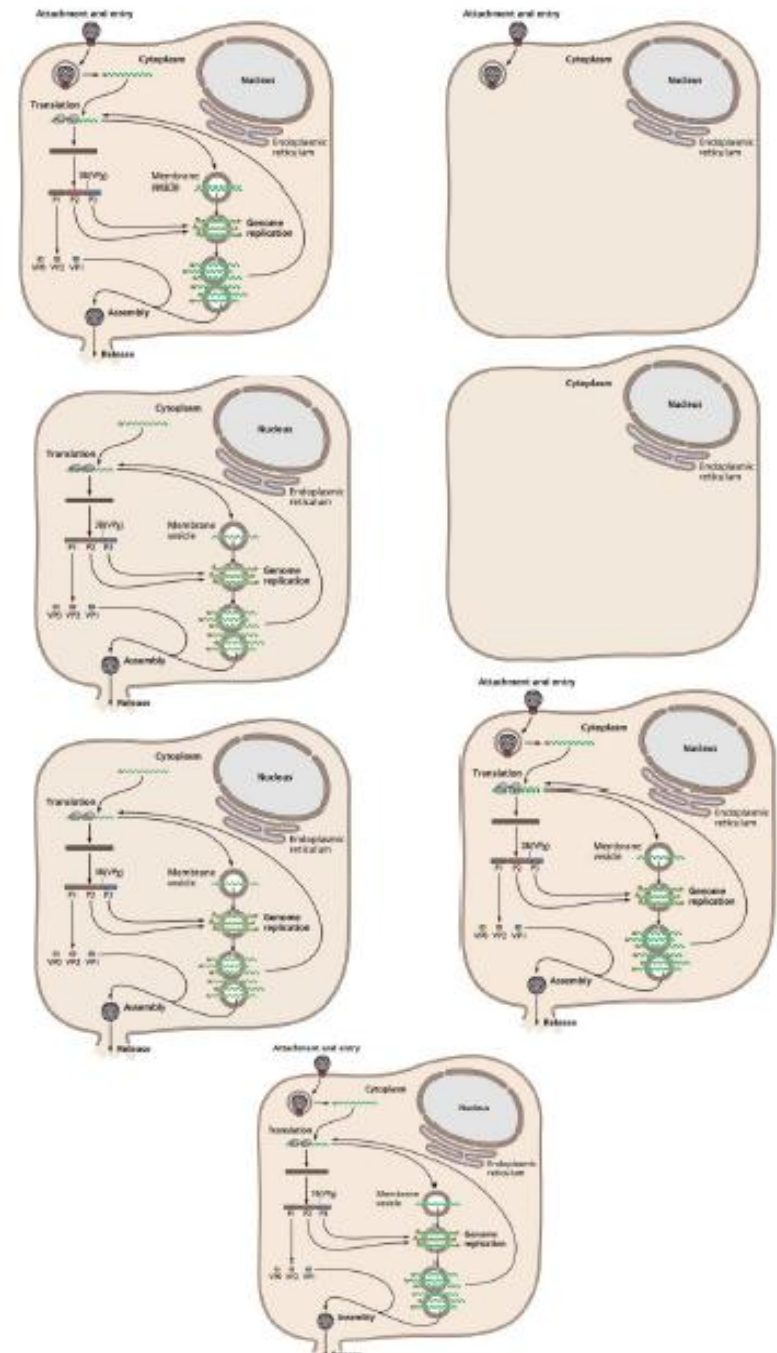


# Cell Culture

# Some important definitions

- A **Susceptible** cell has a functional receptor for a given virus – *the cell may or may not be able to support viral replication*
- A **resistant** cell has no receptor – *it may or may not be competent to support viral replication*
- A **permissive** cell has the capacity to replicate virus – *it may or may not be susceptible*
- A **susceptible AND permissive** is the only cell that can take up a virus particle and replicate it



# Cell culture

Cell culture refers to the process by which cells are grown in a controlled artificial environment. Cells can be maintained in vitro outside of their original body by this process

## Types of animal cell culture

Based on the number of cell division, cell culture can be classified as primary cell culture and cell lines. Cell lines can undergo finite or infinite cell divisions.

## Animal cell culture

### A/ Primary cell culture

This is the cell culture obtained straight from the cells of a host tissue.

The cells dissociated from the parental tissue are grown on a suitable container and the culture thus obtained is called primary cell culture.

Such culture comprises mostly heterogeneous cells and most of the cells divide only for a limited time. However, these cells are much similar to their parents, Depending on their origin, primary cells grow either as an adherent monolayer or in a suspension.



## Adherent cells

These cells are anchorage and propagate as a monolayer. These cells need to be attached to a solid or semi-solid substrate for proliferation.. Fibroblasts and epithelial cells are of such types. When the bottom of the culture vessel is covered with a continuous layer of cells, usually one cell in thickness, these are known as monolayer cultures. Majority of continuous cell lines grow as monolayers. As being single layers, such cells can be transferred directly to a cover slip to examine under microscope.

## Suspension cells

Suspension cells do not attach to the surface of the culture vessels.

These cells are also called anchorage independent or non-adherent cells

which can be grown floating in the culture medium. Hematopoietic stem

cells (derived from blood, spleen and bone marrow) and tumor cells can

be grown in suspension. These cells grow much faster which do not

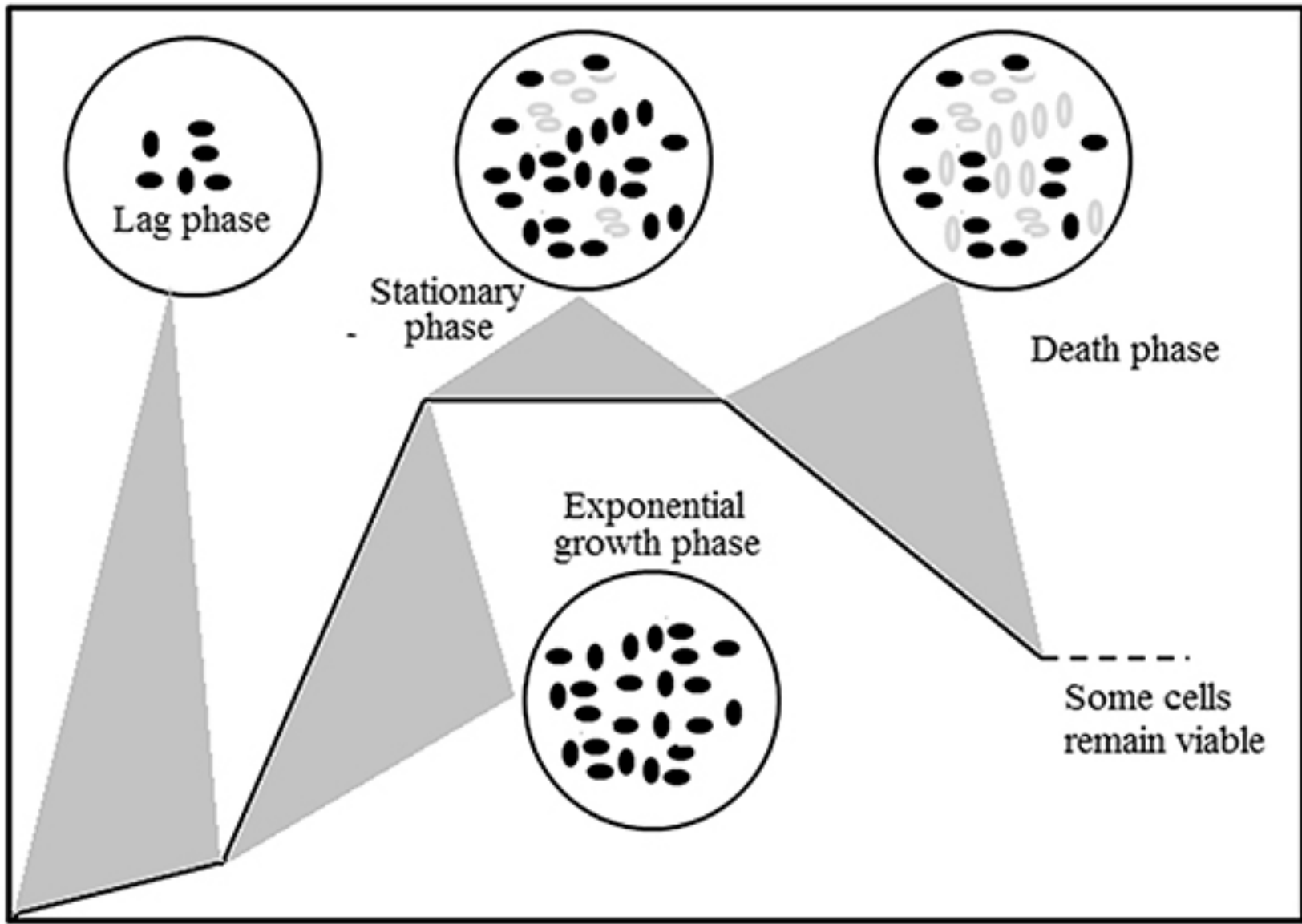
require the frequent replacement of the medium and can be easily

maintained. These are of homogeneous types and enzyme treatment is

not required for the dissociation of cells; similarly these cultures have

short lag period.

LOG OF VIABLE CELLS



Lag phase

Stationary phase

Death phase

Exponential growth phase

Some cells remain viable

HOURS



## Adherent Culture

Appropriate for most cell types, including primary cultures.

Requires periodic passaging, but allows easy visual inspection under inverted microscope.

Cells are dissociated enzymatically (e.g., TrypLE™ Express, trypsin) or mechanically.

Growth is limited by surface area, which may limit product yields.

Requires tissue-culture treated vessel.

Used for cytology, harvesting products continuously, and many research applications.

## Suspension Culture

Appropriate for cells adapted to suspension culture and a few other cell lines that are nonadhesive (e.g., hematopoietic).

Easier to passage, but requires daily cell counts and viability determination to follow growth patterns; culture can be diluted to stimulate growth.

Does not require enzymatic or mechanical dissociation.

Growth is limited by concentration of cells in the medium, which allows easy scale-up.

Can be maintained in culture vessels that are not tissue-culture treated, but requires agitation (i.e., shaking or stirring) for adequate gas exchange.

Used for bulk protein production, batch harvesting, and many research applications.



## Confluent culture and the necessity of sub-culture

After the cells are isolated from the tissue and proliferated under the appropriate conditions, they occupy all of the available substrate i.e. reach confluence. For a few days it can become too crowded for their container and this can be detrimental to their growth, generally leading to cell death if left for long time. The cells thus have to be subculture i.e. a portion of cells is transferred to a new vessel with fresh growth medium which provides more space and nutrients for continual growth of both portions of cells. Hence subculture keeps cells in healthy and in growing state.

A passage number refers specifically to how many times a cell line has been sub-cultured. In contrast with the population doubling level in that the specific number of cells involved is not relevant. It simply gives a general indication of how old the cells may be for various assays.

- Subculture-----passage
- Mouse tail fibroblast culture----5-7 passage
- Hela cell culture-----30-50 passage
- as these are the most similar to the original line  
because at higher passages the cells usually lose their identity and express abnormal proteins.

## B/ Secondary cell culture and cell line

When a primary culture is sub-cultured, it is known as secondary culture or cell line or sub-clone. The process involves removing the growth media and disassociating the adhered cells (usually enzymatically).

Sub-culturing of primary cells to different divisions leads to the generation of cell lines. During the passage, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population. However, as they are sub-cultured serially, they become different from the original cell. On the basis of the life span of culture, the cell lines are categorized into two types:

### ❖ Finite cell lines

The cell lines which go through a limited number of cell division having a limited life span are known as finite cell lines. The cells passage several times and then lose their ability to proliferate, which is a genetically determined event known as senescence. Cell lines derived from primary cultures of normal cells are finite cell lines.

### ❖ Continuous cell lines

When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line. Such transformation/mutation can occur spontaneously or can be chemically or virally induced or from the establishment of cell cultures from malignant tissue. Cell cultures prepared in this way can be sub-cultured and grown indefinitely as permanent cell lines and are immortal.

These cells are less adherent, fast growing, less fastidious in their nutritional requirements, able to grow up to higher cell density and different in phenotypes from the original tissue. Such cells grow more in suspension. They also have a tendency to grow on top of each other in multilayers on culture-vessel surfaces.

