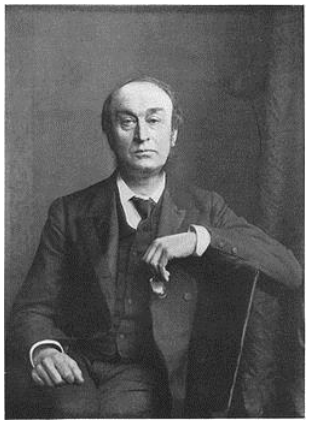
Cell culture

- Cell culture is the process by which cells are grown under controlled conditions, generally outside of their natural environment.

History



The 19th-century English physiologist **Sydney Ringer** developed salt solutions containing the chlorides of sodium, potassium, calcium and magnesium suitable for maintaining the beating of an isolated animal heart outside of the body.

In 1885, **Wilhelm Roux** removed a portion of the medullary plate of an embryonic chicken and maintained it in a warm saline solution for several days, establishing the principle of tissue culture.

Ross Granville Harrison, working at Johns Hopkins Medical School and then at Yale University, published results of his experiments from 1907 to 1910, establishing the methodology of tissue culture.

History

- Cell culture techniques were advanced significantly in the 1940s and 1950s to support research in virology. Growing viruses in cell cultures allowed preparation of purified viruses for the manufacture of vaccines.
- The injectable polio vaccine developed by **Jonas Salk** was one of the first products mass-produced using cell culture techniques.
- This vaccine was made possible by the cell culture research of **John Franklin Enders**, **Thomas Huckle Weller**, and **Frederick Chapman Robbins**, who were awarded a Nobel Prize for their discovery of a method of growing the virus in monkey kidney cell cultures.

What can we do with cells?

- Test pharmaceutical drugs
- Watch disease mechanisms
  - Design potential treatments
- Observe the regenerative process
  - How do cells and tissues repair themselves after damage from illness or injury?
- Observe the developmental process

Technique and instrument

Laminar flow

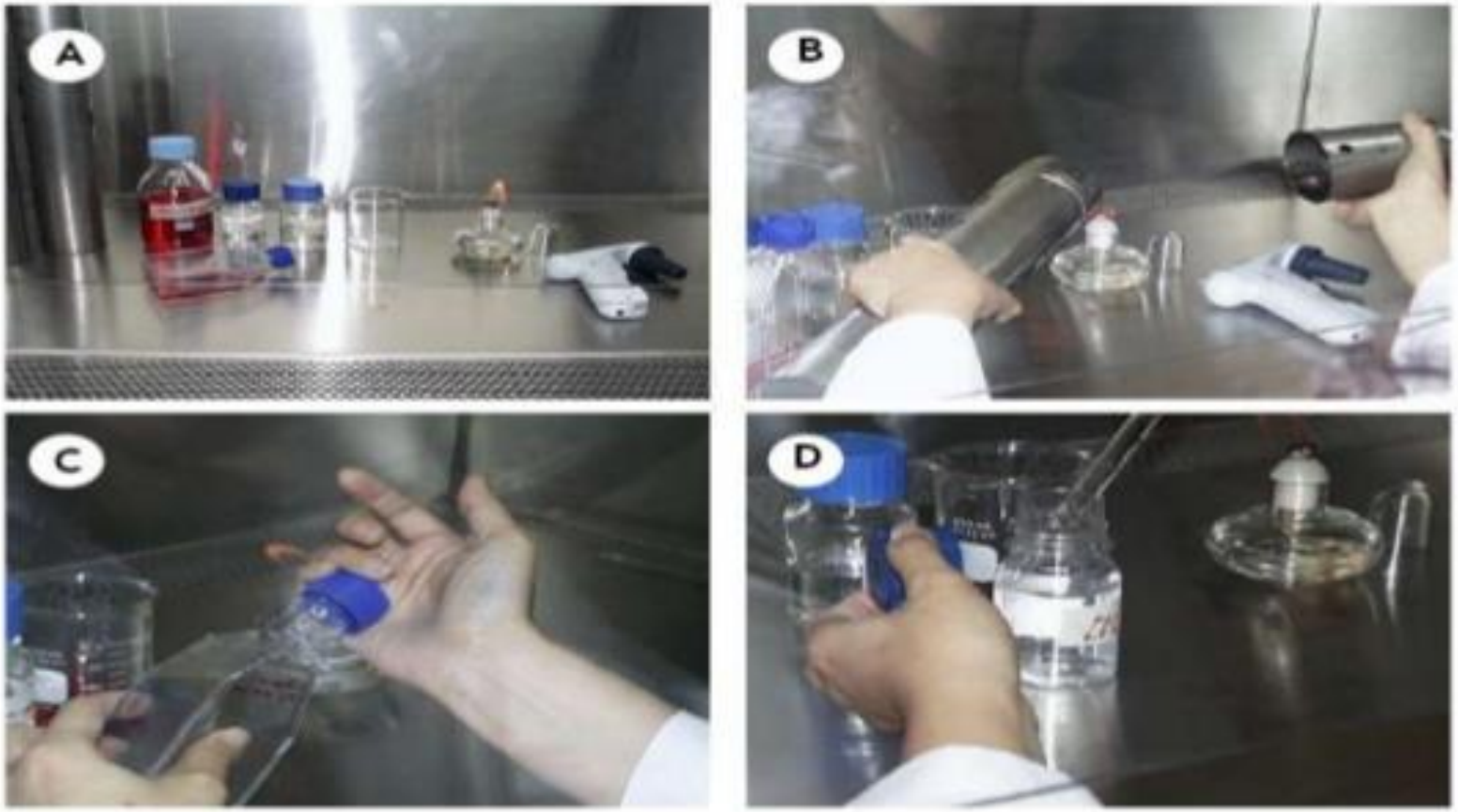


Figure 8. Basic aseptic techniques. A: Performing all maneuvers in a laminar flow hood. B: Using flames to fix microorganisms on container necks. C: Holding a bottle cap with the little finger. D: Avoid touching tops of open vessels while transferring their content.

Carbon dioxide incubator

Figure 1. A CO_2 controlled incubator.

Tissue culture Ware

Figure 3. The disposable plasticware used for culturing cells. A: Multi-well plate (6 wells). B: Flask (75 cm^2).

Culture Media Sterilization

Figure 4. Media sterilization through a 0.22 µm membrane filter assembled in a filter holder.

Cell counting

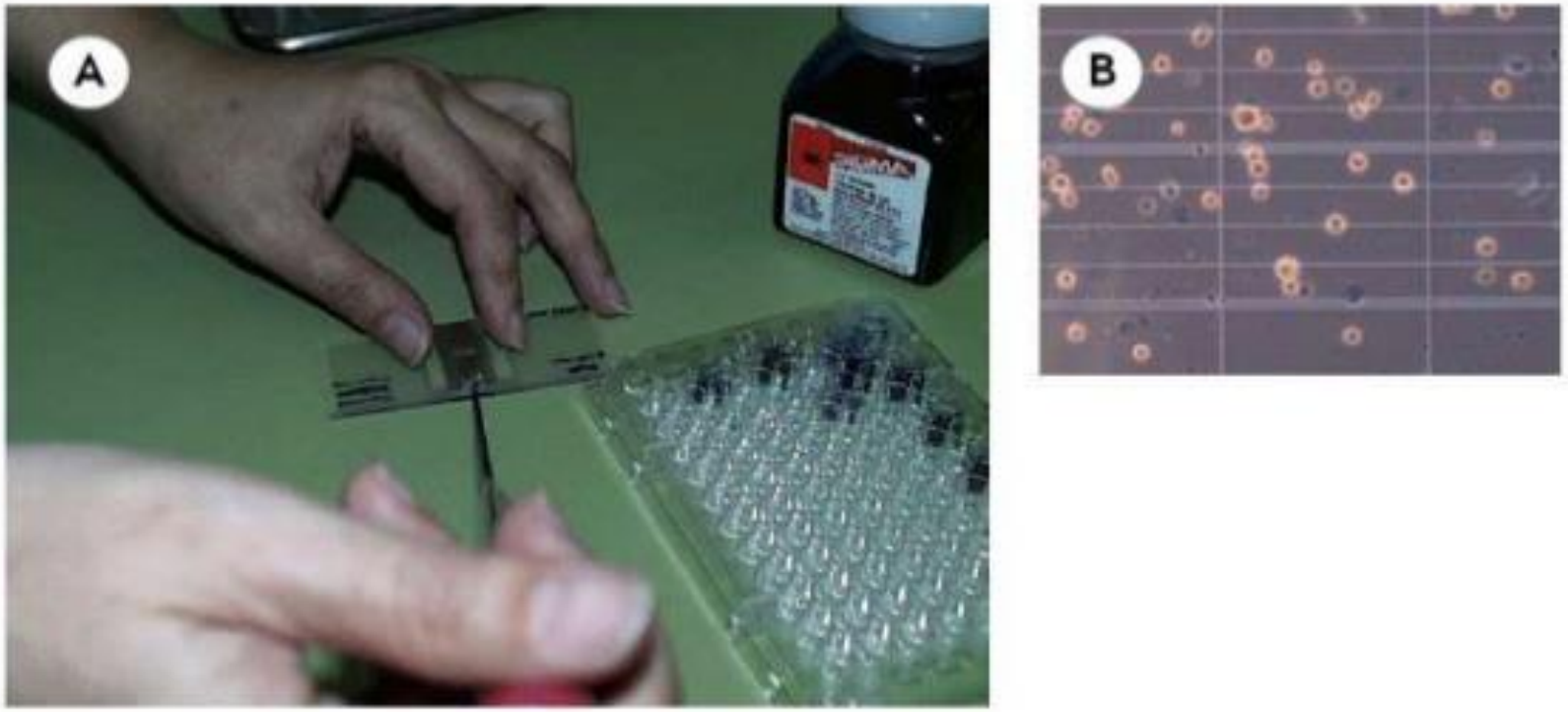


Figure 9. Quantitation of cell viability by trypan blue exclusion. A: Filling the counting chamber with a trypan blue-stained cell suspension. B: Counting viable and non-viable cells under a 100x microscopic field.

Isolation of cells

- Cells can be isolated from tissues for *ex vivo* culture in several ways.
- Cells can be easily purified from blood;

  However, only the white cells are capable of growth in culture.
- Mononuclear cells can be isolated from soft tissues by enzymatic digestion with enzymes such as collagenase, trypsin, or pronase, which break down the extracellular matrix.
- Alternatively, pieces of tissue can be placed in growth media, and the cells that grow out are available for culture. This method is known as *explant culture.*

Cells that are cultured directly from a subject are known as primary cells. With the exception of some derived from tumors, most primary cell cultures have limited lifespan.

An established or immortalized cell line has acquired the ability to proliferate indefinitely either through random mutation or deliberate modification, such as artificial expression of the telomerase gene.

Hayflick limit (or **Hayflick phenomenon**)

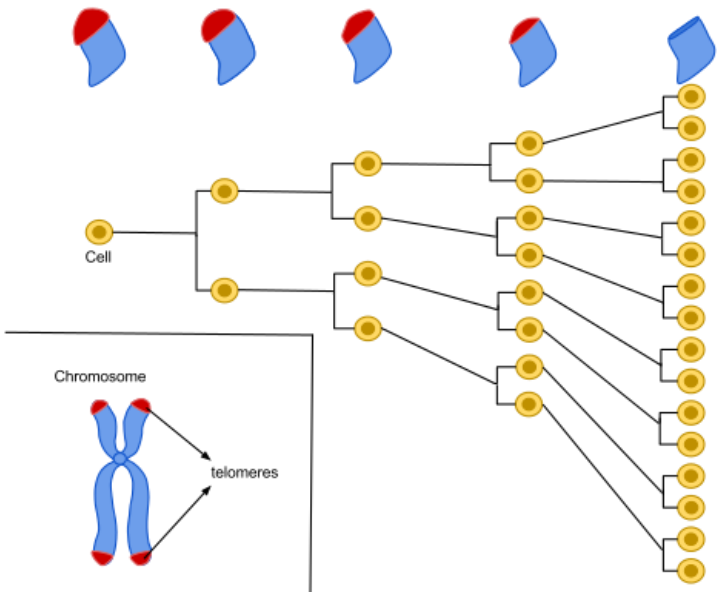
- For the majority of isolated primary cells, they undergo the process of senescence and stop dividing after a certain number of population doublings while generally retaining their viability (described as the *Hayflick limit*).

The **Hayflick limit** is the number of times a normal human cell population will divide until cell division stops. Empirical evidence shows that the telomeres associated with each cell's DNA will get slightly shorter with each new cell division until they shorten to a critical length.

Cell phases

- Hayflick describes three phases in the life of a cell. At the start of his experiment he named the primary culture "*phase one*".
- *Phase two* is defined as the period when cells are proliferating – Hayflick called it the time of "luxuriant growth".
- After months of doubling the cells eventually reach *phase three*, a phenomenon of senescence – cell growth diminishes and then cell division stops altogether.

Hayflick limit



- The average cell will divide between 50-70 times before cell death. As the cell divides the telomeres on the end of the chromosome get smaller.

The Hayflick Limit is the theory that due to the telomeres shortening through each division, the telomeres will eventually no longer be present on the chromosome. This end stage is known as senescence and proves the concept that links the deterioration of telomeres and aging.

Maintaining cells in culture

- Cells are grown and maintained at an appropriate temperature and gas mixture (typically, 37 °C, 5% CO_2 for mammalian cells) in a cell incubator.

Culture conditions vary widely for each cell type, and variation of conditions for a particular cell type can result in different phenotypes.

Growth Medium

- Aside from temperature and gas mixture, the most commonly varied factor in culture systems is the cell growth medium.
- Recipes for growth media can vary in pH, glucose concentration, growth factors, and the presence of other nutrients.

Growth Medium

The growth factors used to supplement media are often derived from the serum of animal blood, such as fetal bovine serum (FBS), bovine calf serum, equine serum, and porcine serum.

One complication of these blood-derived ingredients is the potential for contamination of the culture with viruses or prions, particularly in medical biotechnology applications.

Current practice is to minimize or eliminate the use of these ingredients wherever possible and use human platelet lysate (hPL). This eliminates the worry of cross-species contamination when using FBS with human cells. hPL has emerged as a safe and reliable alternative as a direct replacement for FBS or other animal serum.

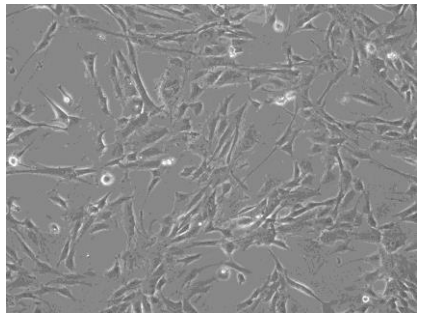
Plating density

- **Plating density** (number of cells per volume of culture medium) plays a critical role for some cell types.
- For example, a lower plating density makes granulosa cells exhibit estrogen production, while a higher plating density makes them appear as progesterone-producing theca lutein cells

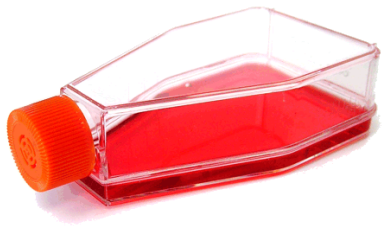
*Cells can be grown either in **suspension** or adherent cultures.*

- Some cells naturally live in suspension, without being attached to a surface, such as cells that exist in the bloodstream.
- There are also cell lines that have been modified to be able to survive in suspension cultures so they can be grown to a higher density than adherent conditions would allow.

Cells can be grown either in suspension or ***adherent cultures.***



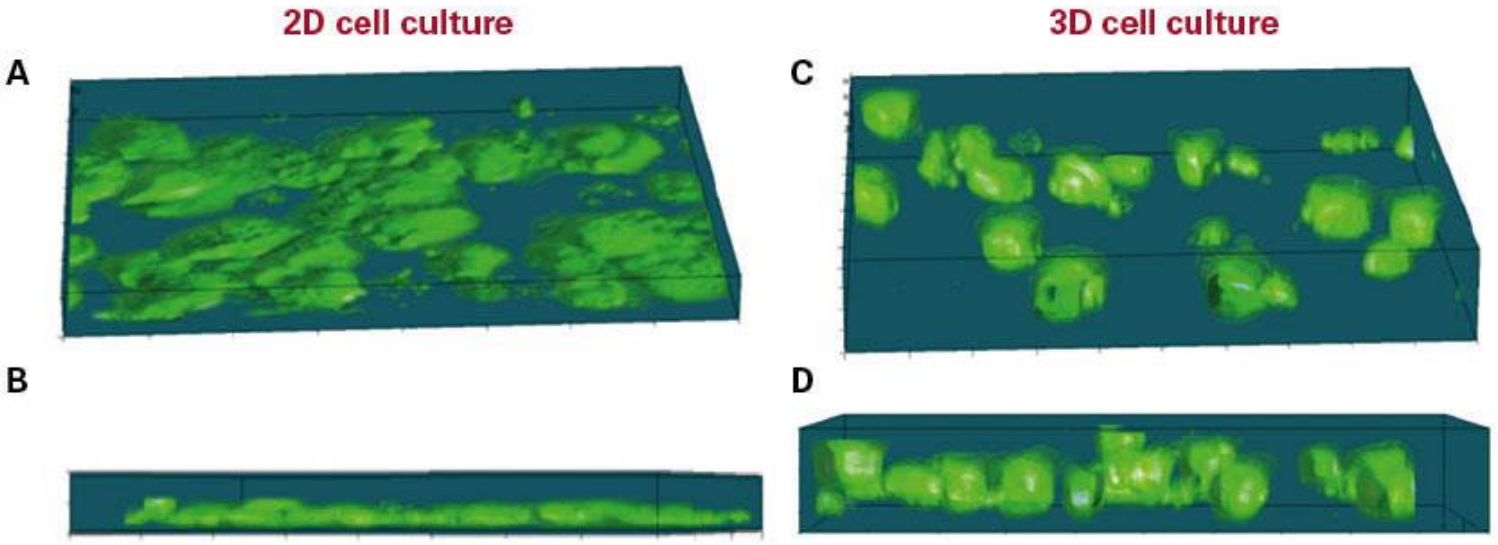
- Adherent cells require a surface, such as tissue culture plastic, which may be coated with extracellular matrix (such as collagen and laminin) components to increase adhesion properties and provide other signals needed for growth and differentiation.



- Most cells derived from solid tissues are adherent.

3D cell culture

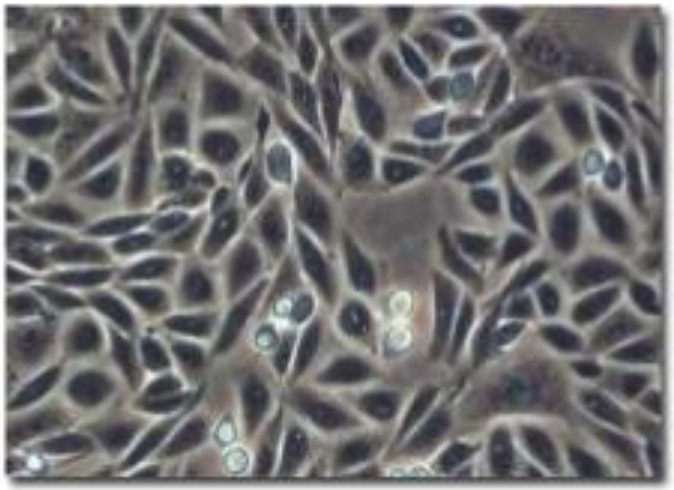
- Another type of adherent culture is organotypic culture, which involves growing cells in a three-dimensional (3-D) environment as opposed to two-dimensional culture dishes.
- This 3D culture system is biochemically and physiologically more similar to *in vivo* tissue, but is technically challenging to maintain because of many factors (e.g. diffusion).



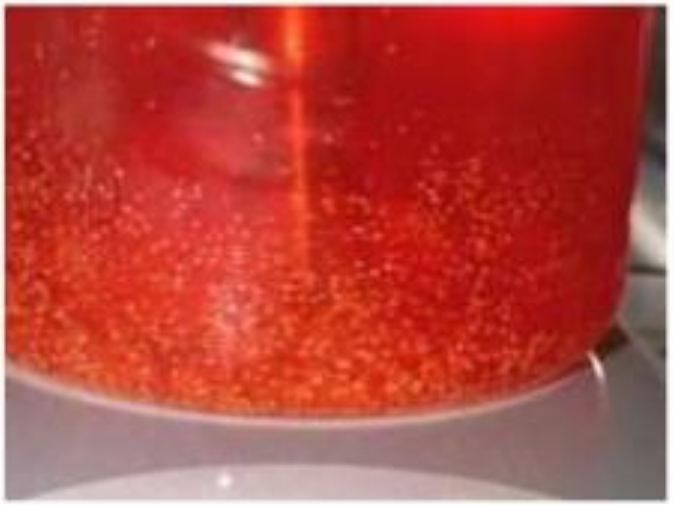
Cells grown on conventional 2D surfaces (A and B) adopt a typical flattened morphology covering a large surface area in horizontal x–y plane (A) and have a reduced height in the vertical z plane (B). In comparison, cells maintained in alvetex® (C and D) retain a more cuboidal morphology and 3D cell structure, particularly in the z-plane.*

Adherent vs Suspension cells for tissue culture

- **Adherent cells**: cells grow in a single layer (called a monolayer) attached to the tissue culture dish
  - Cell growth is limited by available surface area on which cells can grow
  - To passage adherent cells, the cells must be released from the dish (done either enzymatically, chemically, or mechanically)



- **Suspension cells** : cells are suspended in liquid as single cells or as free-floating clumps of a few cells
  - To passage suspension cell cultures, a proportion of the cells in culture are diluted into a larger volume of medium



Manipulation of cultured cells

- Among the common manipulations carried out on culture cells are media changes, passaging cells, and transfecting cells.
- These are generally performed using tissue culture methods that rely on aseptic technique. Aseptic technique aims to avoid contamination with bacteria, yeast, or other cell lines.
- Manipulations are typically carried out in a biosafety hood or laminar flow cabinet to exclude contaminating micro-organisms. Antibiotics (e.g. penicillin and streptomycin) and antifungals (e.g.amphotericin B) can also be added to the growth media.
- As cells undergo metabolic processes, acid is produced and the pH decreases. Often, a pH indicator is added to the medium to measure nutrient depletion.

Media changes

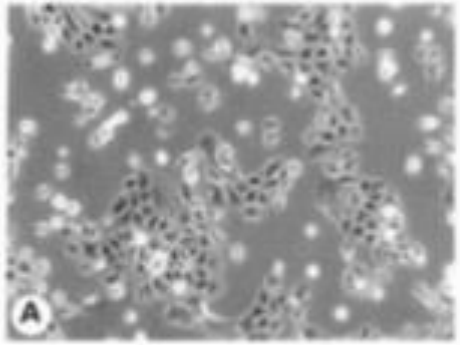
- In the case of adherent cultures, the media can be removed directly by aspiration, and then is replaced. Media changes in non-adherent cultures involve centrifuging the culture and resuspending the cells in fresh media.

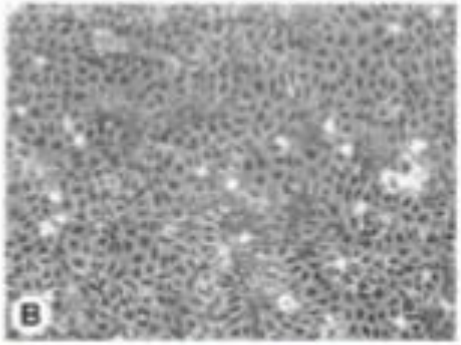
Passaging cells

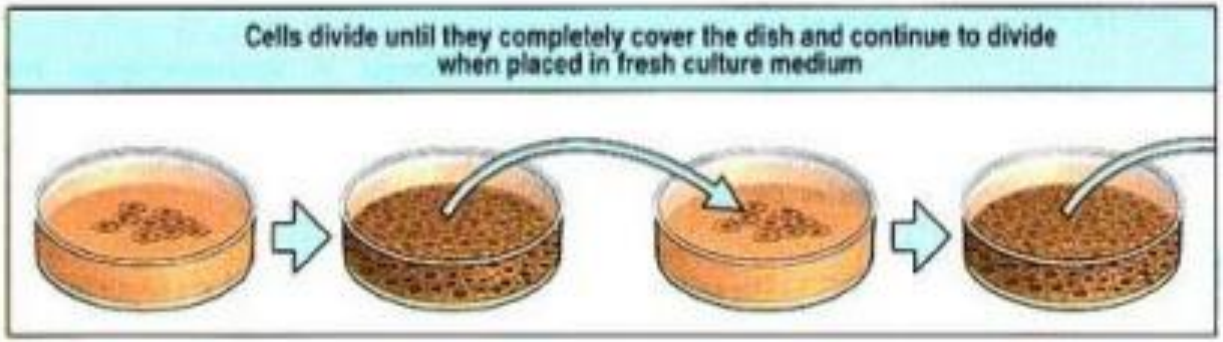
- Passaging (also known as subculture or splitting cells) involves transferring a small number of cells into a new vessel.
- Cells can be cultured for a longer time if they are split regularly, as it avoids the senescence associated with prolonged high cell density.
- Suspension cultures are easily passaged with a small amount of culture containing a few cells diluted in a larger volume of fresh media.
- For adherent cultures, cells first need to be detached; this is commonly done with a mixture of trypsin-EDTA; however, other enzyme mixes are now available for this purpose. A small number of detached cells can then be used to seed a new culture. Some cell cultures, such as RAW cells are mechanically scraped from the surface of their vessel with rubber scrapers.

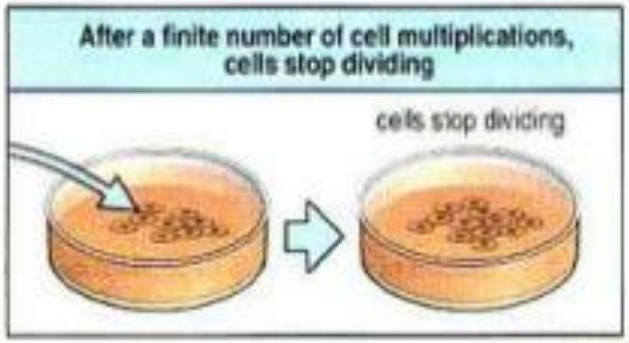
Passaging cells (subculturing cells)

- Process of diluting cell number in order to keep cells actively growing
- For adherent cells, when they cover the tissue culture dish, they need to passaged
  - Otherwise, the cells will become unhealthy and stop growing









Transfection and transduction

- Another common method for manipulating cells involves the introduction of foreign DNA by transfection. This is often performed to cause cells to express a protein of interest.
- More recently, the transfection of RNAi constructs have been realized as a convenient mechanism for suppressing the expression of a particular gene/protein.
- DNA can also be inserted into cells using viruses, in methods referred to as transduction, infection or transformation.
- Viruses, as parasitic agents, are well suited to introducing DNA into cells, as this is a part of their normal course of reproduction.

Cell counting

- **Cell counting** is any of various methods for the counting or similar quantification of cells in the life sciences, including medical diagnosis and treatment. It is an important subset of cytometry, with applications in research and clinical practice. For example, the complete blood count can help a physician to determine why a patient feels unwell and what to do to help. Cell counts within liquid media (such as blood, plasma, lymph, or laboratory rinsate) are usually expressed as a number of cells per unit of volume, thus expressing a concentration (for example, 5,000 cells per milliliter).