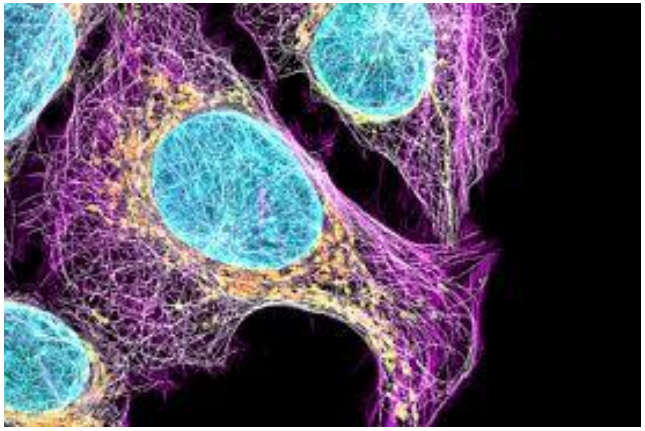
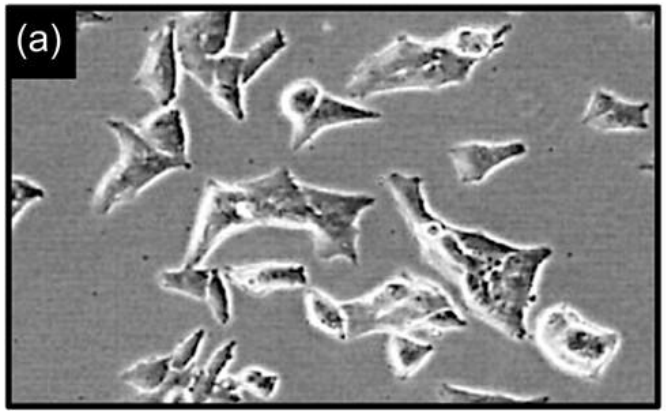
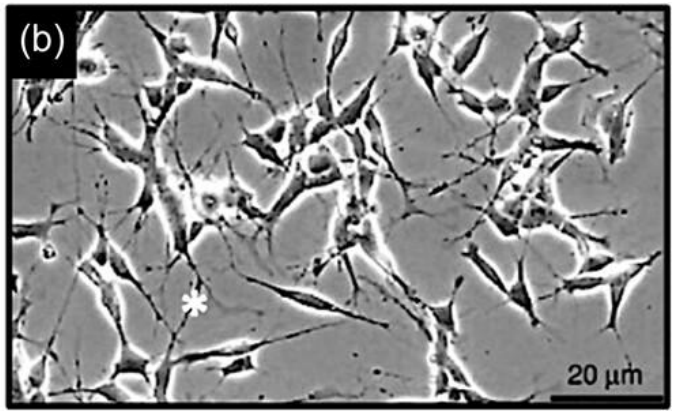






https://microbenotes.com/animal-cell-culture/

Introduction

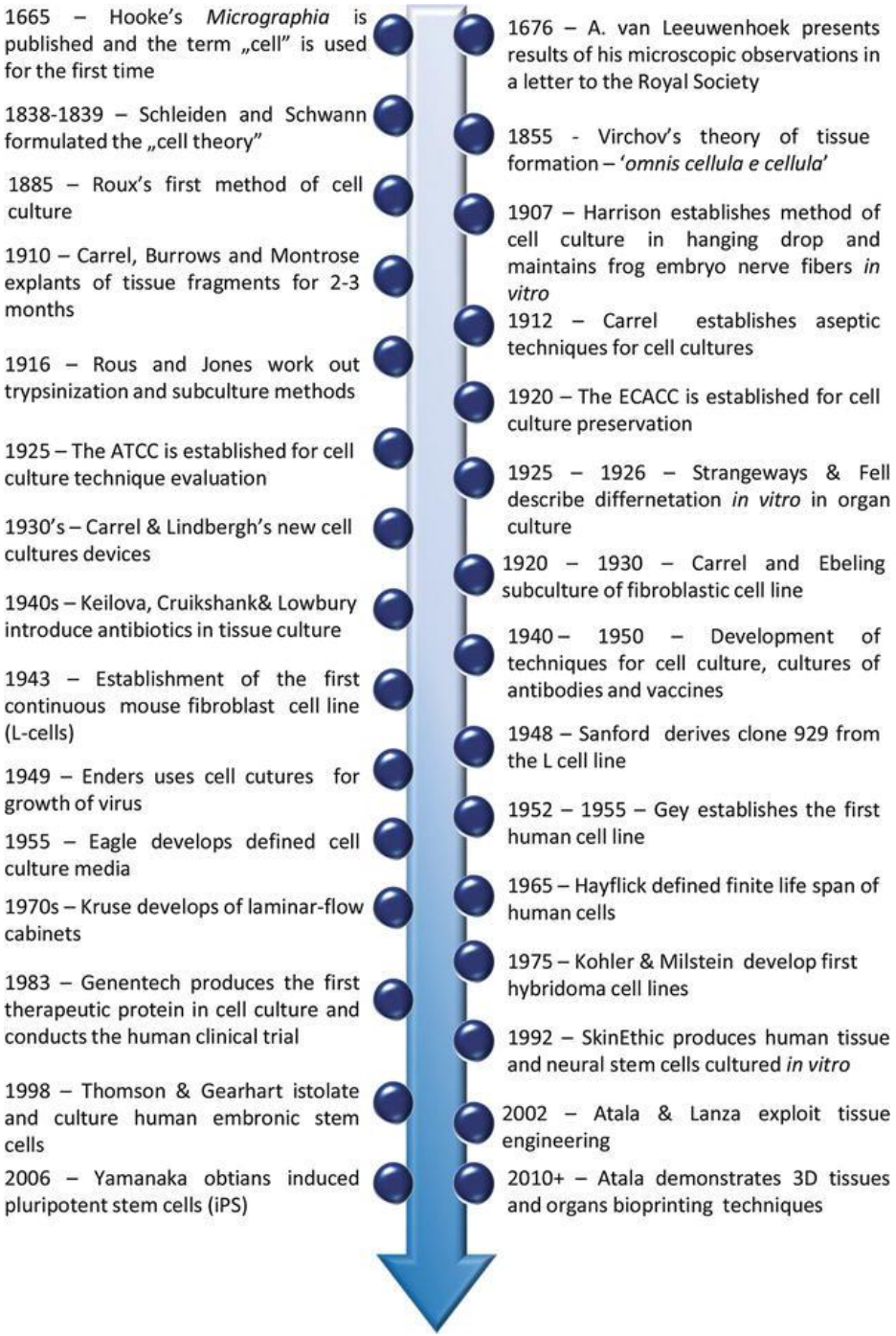
In vitro **cell cultures:**

- cells **isolated from their environment**

- enabled to live **within a defined system**

- fundamental tool for biochemical, microbiological, pharmacological studies …

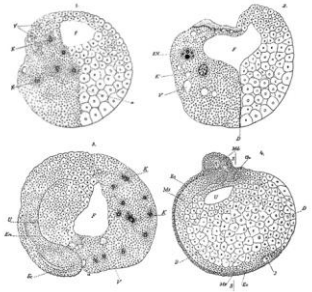
- **useful in the production** of growth factors, monoclonal antibodies, vaccines, recombinant proteins in general …

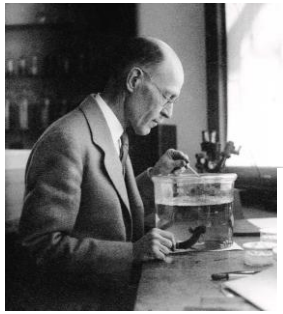

1665 – Hooke's *Micrographia* is published and the term „cell" is used for the first time
1676 – A. van Leeuwenhoek presents results of his microscopic observations in a letter to the Royal Society
1838-1839 – Schleiden and Schwann formulated the „cell theory"
1855 - Virchov's theory of tissue formation – '*omnis cellula e cellula*'
1885 – Roux's first method of cell culture
1907 – Harrison establishes method of cell culture in hanging drop and maintains frog embryo nerve fibers *in vitro*
1910 – Carrel, Burrows and Montrose explants of tissue fragments for 2-3 months
1912 – Carrel establishes aseptic techniques for cell cultures
1916 – Rous and Jones work out trypsinization and subculture methods
1920 – The ECACC is established for cell culture preservation
1925 – The ATCC is established for cell culture technique evaluation
1925 – 1926 – Strangeways & Fell describe differnetation *in vitro* in organ culture
1930's – Carrel & Lindbergh's new cell cultures devices
1920 – 1930 – Carrel and Ebeling subculture of fibroblastic cell line
1940s – Keilova, Cruikshank& Lowbury introduce antibiotics in tissue culture
1940 – 1950 – Development of techniques for cell culture, cultures of antibodies and vaccines
1943 – Establishment of the first continuous mouse fibroblast cell line (L-cells)
1948 – Sanford derives clone 929 from the L cell line
1949 – Enders uses cell cutures for growth of virus
1952 – 1955 – Gey establishes the first human cell line
1955 – Eagle develops defined cell culture media
1965 – Hayflick defined finite life span of human cells
1970s – Kruse develops of laminar-flow cabinets
1975 – Kohler & Milstein develop first hybridoma cell lines
1983 – Genentech produces the first therapeutic protein in cell culture and conducts the human clinical trial
1992 – SkinEthic produces human tissue and neural stem cells cultured *in vitro*
1998 – Thomson & Gearhart istolate and culture human embronic stem cells
2002 – Atala & Lanza exploit tissue engineering
2006 – Yamanaka obtians induced pluripotent stem cells (iPS)
2010+ – Atala demonstrates 3D tissues and organs bioprinting techniques

Cell culture is the process by which prokaryotic, eukaryotic or plant cells are grown under controlled conditions.

But **in practice** it refers to the culturing of cells derived from **animal cells**.

Roux in **1885** for the first time maintained embryonic chick cells in a cell culture





Cell culture was first successfully undertaken by Ross Harrison in **1907**

EXPERIMENTS IN TRANSPLANTING LIMBS AND THEIR BEARING UPON THE PROBLEMS OF THE DEVELOPMENT OF NERVES[1]

BY

ROSS GRANVILLE HARRISON

WITH FOURTEEN FIGURES

Several years ago Braus described a series of ingenious experiments in transplanting limbs of amphibian (Bombinator) larvæ. The experiments were made mainly for the purpose of inquiring into questions relative to the development of peripheral nerves and their author has interpreted his results in accordance with Hensen's theory. Briefly stated this theory is that the nerve cen-

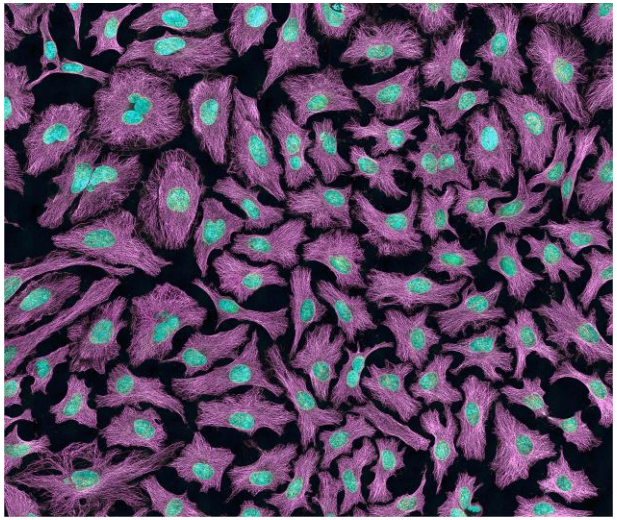
1940s: The use of the antibiotics penicillin and streptomycin in culture medium decreased the problem of contamination in cell culture.

Introduction

- **1951** Gey established a **continuous cell line** from a human cervical carcinoma

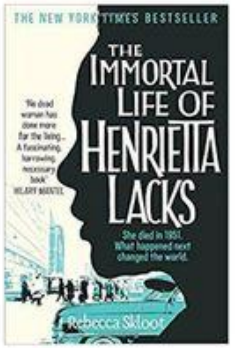
The patients was **He**nrietta **La**cks.

Cell line is known as **HeLa** cells



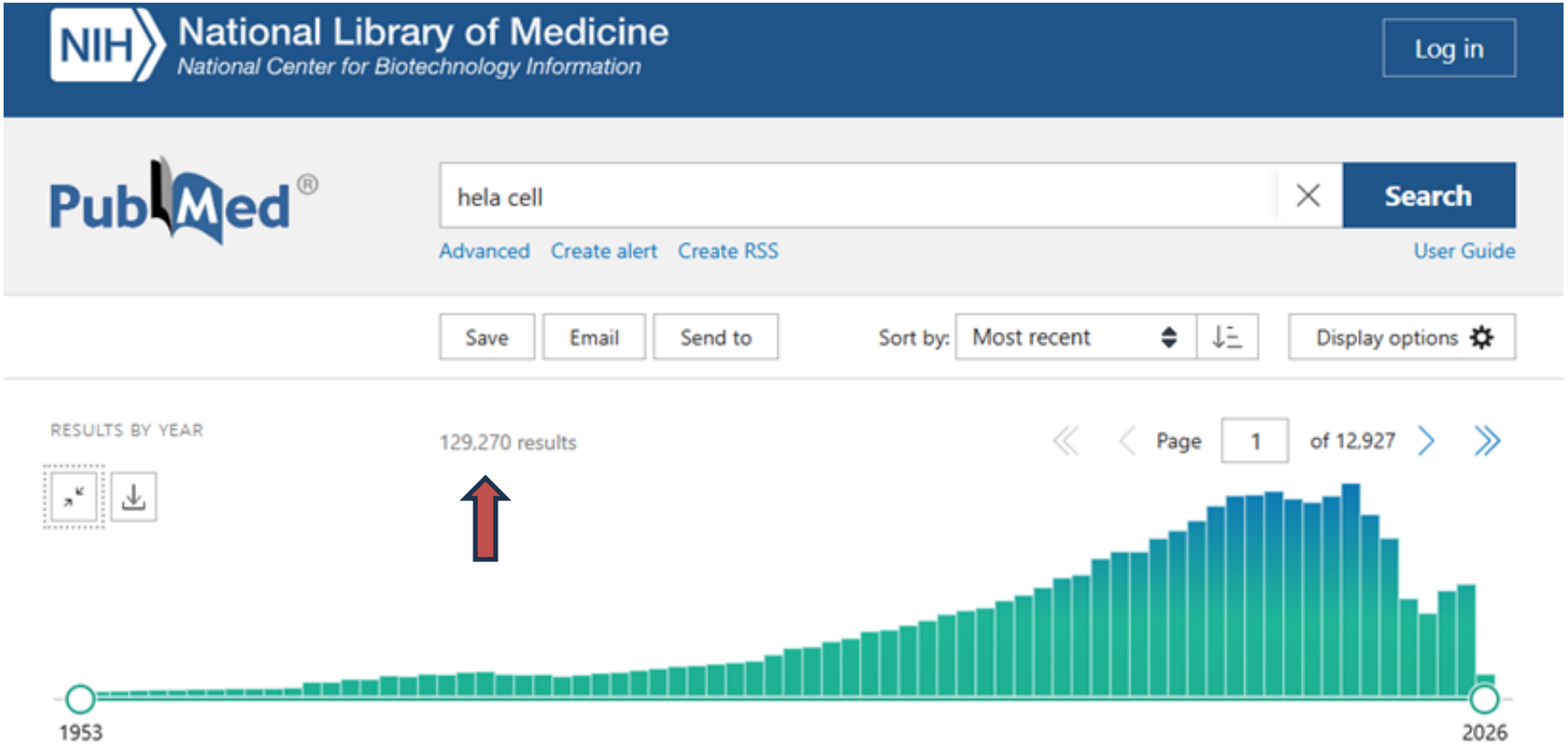
born
Loretta Pleasant in 1920

The immortal life of Henrietta Lacks



- 1951 Tissue were taken without her knowledge
- 1952 HeLa cells were used to develop **polio vaccine**
- 1955 Isolation of a single cell for **cloning**
- 1960 HeLa **went to space** before any astronaut
- 1984 HeLa was used to prove that **HPV infection causes cancer**
- 1986 Mechanism of **HIV infection** were studied
- 1989 **Telomerase** were described in HeLa
- 1993 Tuberculosis was studied
- 2013 Whole genome data of HeLa were published

George Otto Gey


National Library of Medicine
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Most recent
Display options
RESULTS BY YEAR
129,270 results
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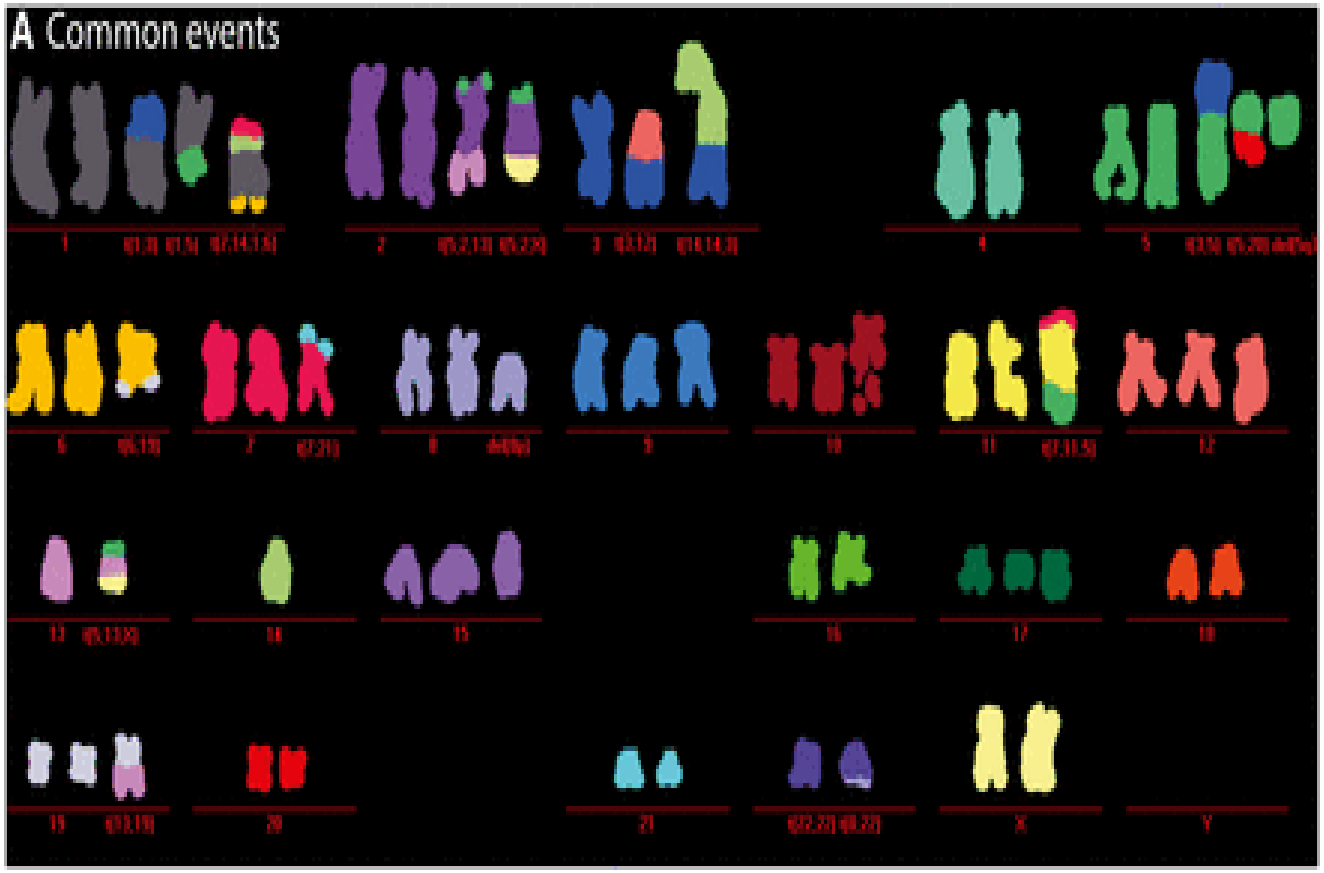
of 12,927
1953
2026

The Genomic and Transcriptomic Landscape of a HeLa Cell Line

Jonathan J. M. Landry,*,[1] Paul Theodor Pyl,*,[1] Tobias Rausch,* Thomas Zichner,* Manu M. Tekkedil,* Adrian M. Stütz,* Anna Jauch,† Raeka S. Aiyar,* Gregoire Pau,*,[2] Nicolas Delhomme,*,[3] Julien Gagneur,*,[4] Jan O. Korbel,* Wolfgang Huber,*,[5] and Lars M. Steinmetz*,[5]

*European Molecular Biology Laboratory, Genome Biology Unit, 69117 Heidelberg, Germany, and †University Hospital Heidelberg, Institute of Human Genetics, 69120 Heidelberg, Germany



- **1955: Eagle** studied **the nutrient requirements** of selected cells in culture and established the first widely used chemically defined medium.

- **1964**: **Littlefield** introduced the **HAT medium** for cell selection.

- **1965**: **Ham** introduced the first **serum-free medium** which was able to support the growth of some cells.

Major development's in cell culture technology

- **First**

the use of **antibiotics** which inhibits the growth of contaminants.

- **Second**

the **use of trypsin** to remove adherent cells to subculture further from the culture vessel

- **Third**

the use of **chemically defined culture medium**.

Cell culture

- *Bacteria*
- *Yeast (lower eukaryotic)*
- *Plant cell Cultures*

- **Primary cultures of animal cells**

- **Cell lines**

- In Suspension

- Adherents

CELL CULTURE LABORATORY

Cell cultures **last longer** and allow a **variety of studies** that are not possible with tissues.

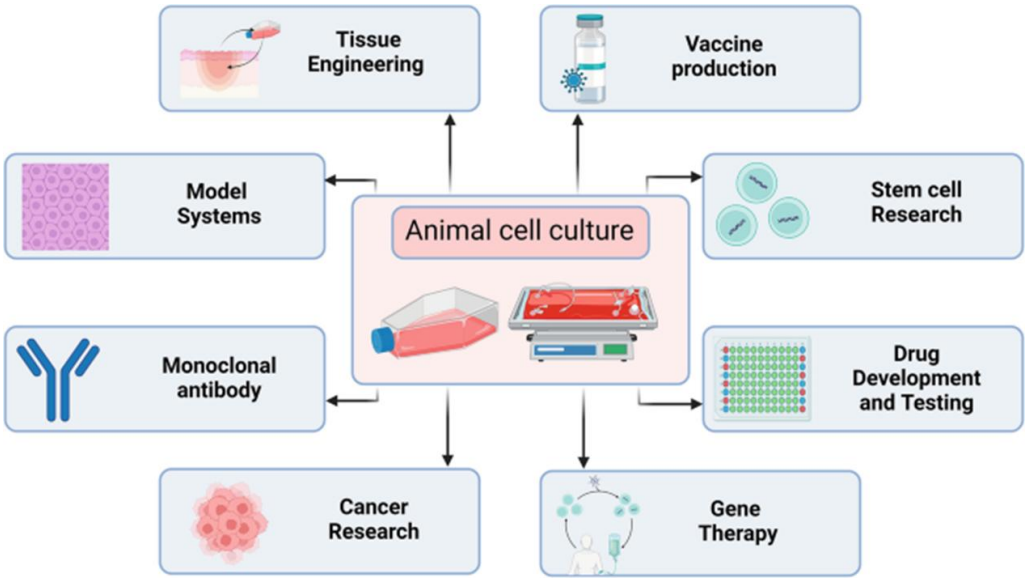
-the study of **cellular differentiation** (cell cycle)

- pathological studies;

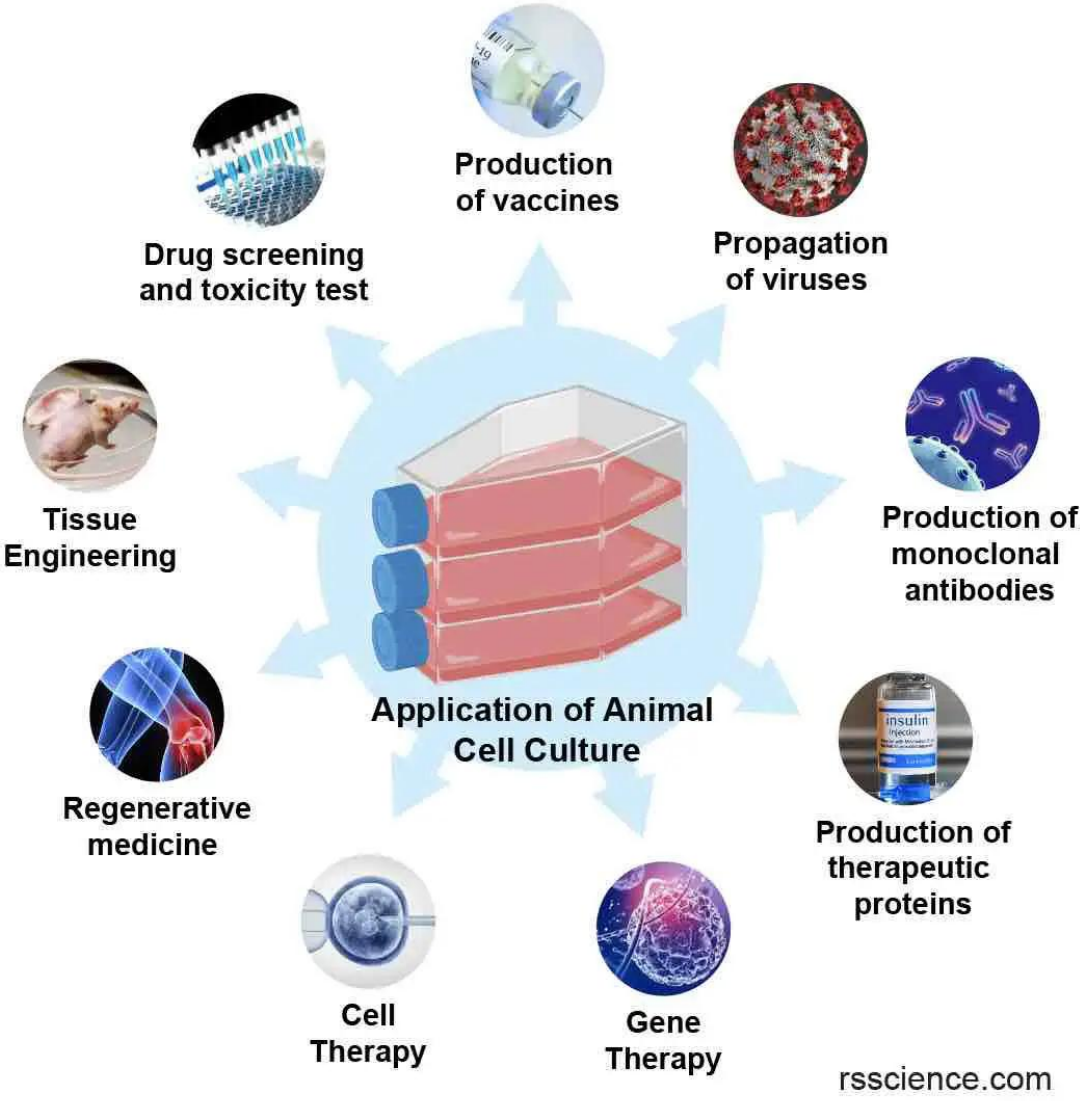
- experimental models in biochemistry, pharmacology, physiology;

- genetic manipulation;

- biotechnological applications



Application of Animal cell culture



Advantages in cell cultures

- **Commercial availability** of media, factors, etc.
- **Documentation** on insulation/preservation
- Control of **environmental variables** (T, PH…)

- Wide availability of **cell types**

- Simplicity in **replicating experiments**
- Possibility of **sizing experiments** with biotechnological purposes
- Reduction in the **number of animals killed**

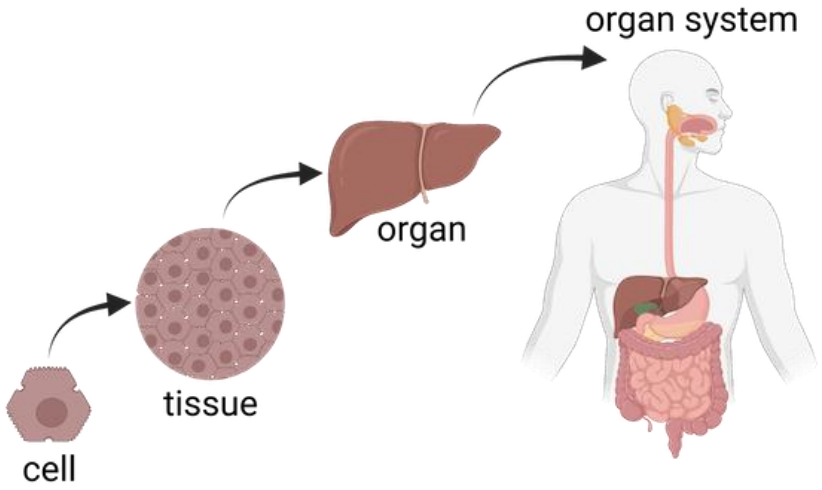
Disadvantages in cell cultures

- **Cost** of materials required (eg, serum, growth factors…)

- Very specialized and **laborious methods**

- **Simplified systems** compared to an integrated body

- Difficulty in correlating ***in vitro*** concentrations with those ***in vivo***



Tipi di colture cellulari

- **Colture in monostrato/ in sospensione**: le cellule tendono ad aderire alla superficie del recipiente di coltura; moltiplicandosi formano un monostrato, in quanto risentono dell'inibizione da contatto. Cellule trasformate possono formare pluristrati.

- **Colture a breve/lungo termine:** nel primo caso il numero di cicli cui vanno incontro le cellule è ridotto; in colture a lungo termine le cellule si dividono molte volte, o addirittura illimitatamente (**linee cellulari stabilizzate**)

- **Colture clonali:** le cellule vengono diluite prima della "semina" in modo da avere ogni cellula separata, che prolifera formando una singola colonia (***clone***).

Monolayer / suspension cultures:

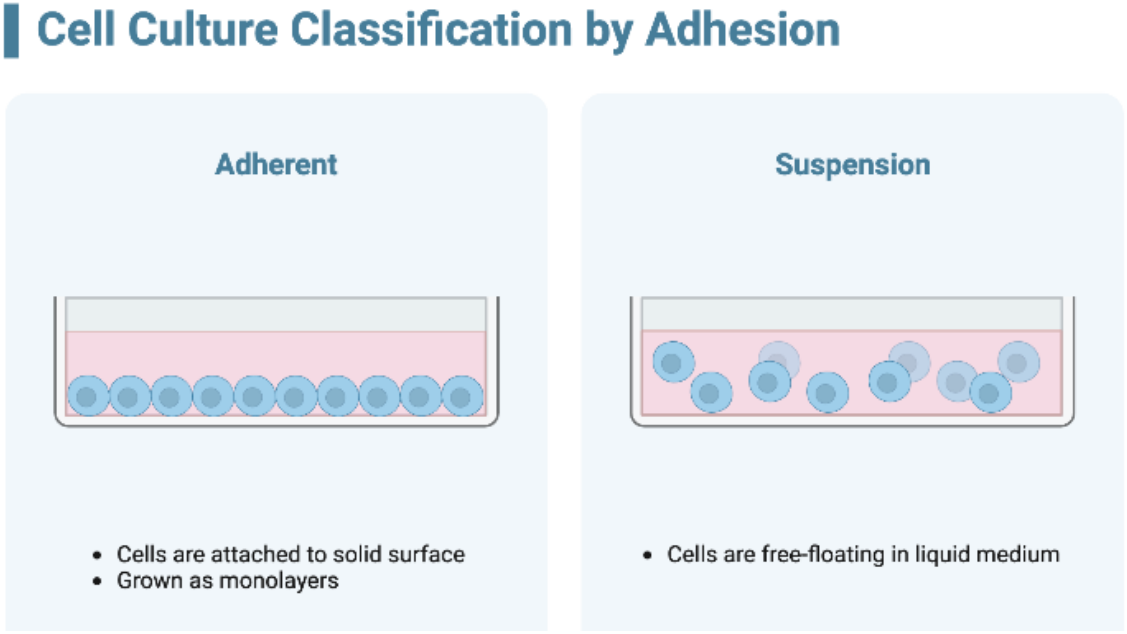
Cells tend to adhere to the surface of the culture vessel; as they multiply, they form a monolayer, as they are sensitive to contact inhibition. Transformed cells can form multilayers.

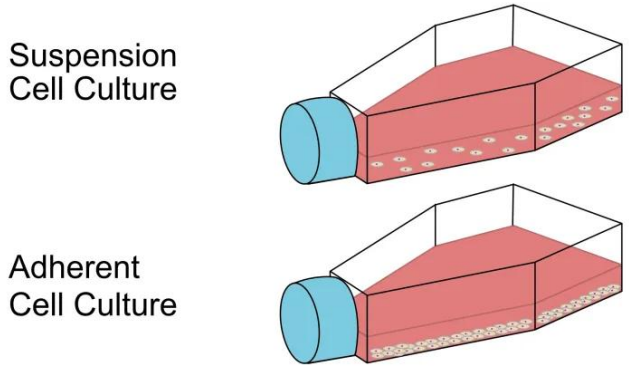
Short-term / long-term cultures:

In the first case, the number of cycles the cells undergo is limited; in long-term cultures, cells divide many times, or even indefinitely (established cell lines).

Culturing of cells

- Cells are cultured as **anchorage dependent** or **independent**



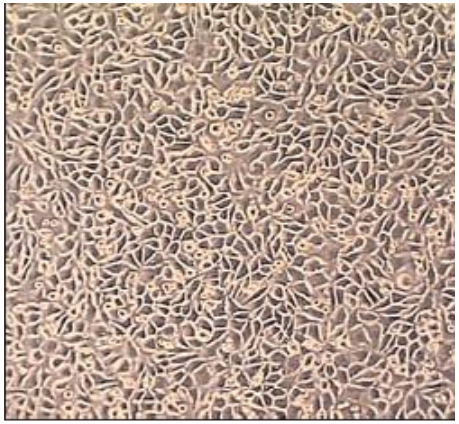


- Cell lines derived from **normal tissues** are considered as **anchorage-dependent** grows only on a suitable substrate e.g. tissue cells

- **Suspension cells** are **anchorage-independent** e.g. blood cells

- **Transformed cell lines** either grows as monolayer or as suspension

Cells that are part of **solid tissues** grow in vitro **adhering** to the surface of the culture plates.



Confluent cells in monolayer L929 mouse fibroblast cells.

The cells of **haematopoietic origin**, which normally grow in fluid medium, grow in suspension and multiply in vitro **without adhering**.

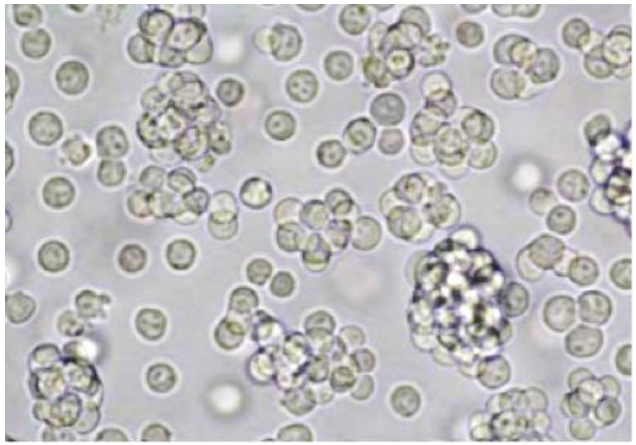
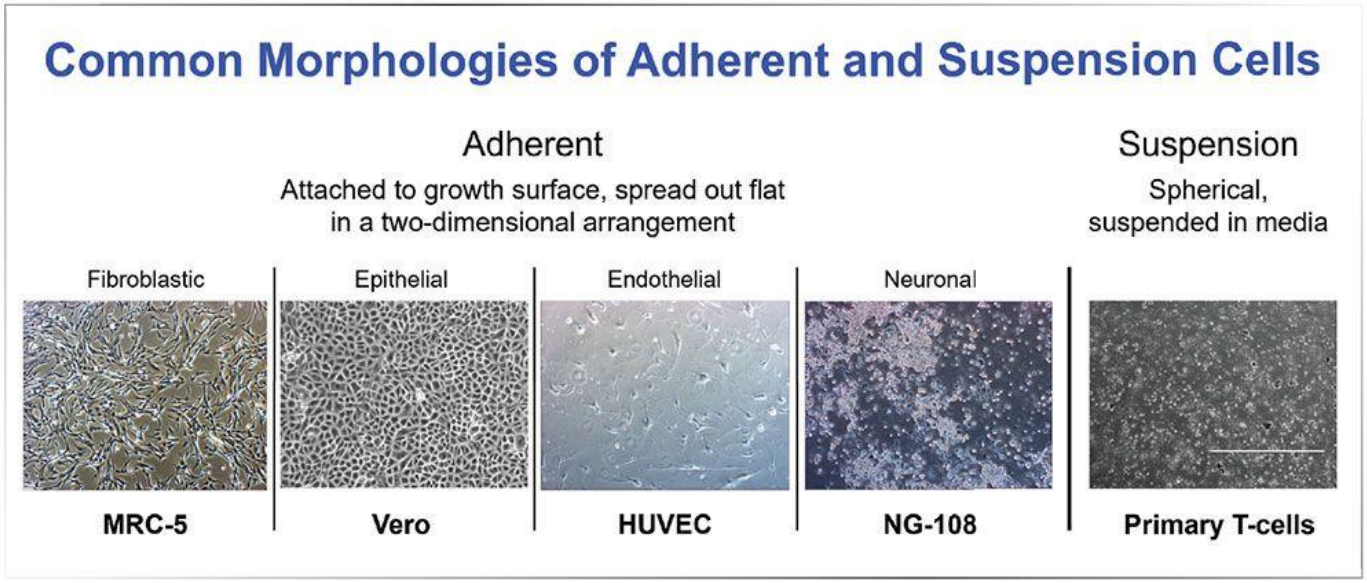


Figure 1. Visual showing the characteristic cell morphologies of adherent and suspension cells.



Monolayer / suspension cultures:

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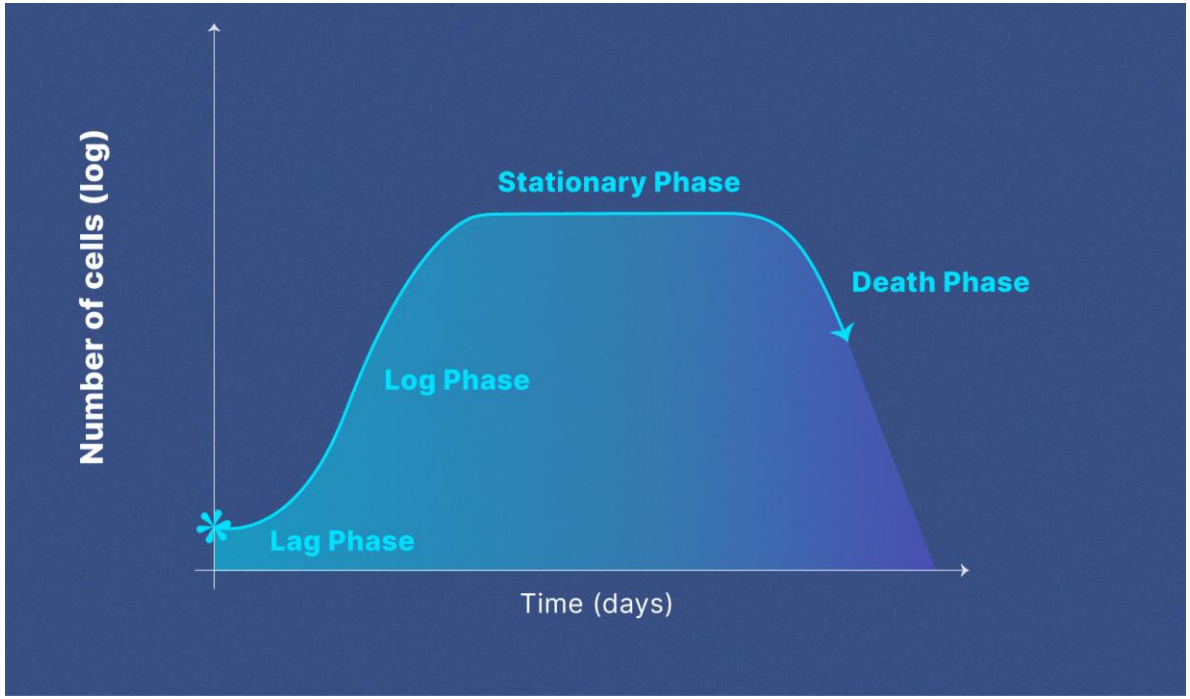
Short-term / long-term cultures:

In the first case, the number of cycles the cells undergo is limited; in long-term cultures, cells divide many times, or even indefinitely (established cell lines).

Cell culture growth generally occurs in **four phases**



Lag phase occurs when cells are acclimatizing to culture conditions and are not dividing.

Log phase occurs when cells are actively dividing. This is the best phase for cell experimentation and data collection.
Cells should be sub-cultured when they reach late log phase.

When cells approach overcrowding, cell growth slows. This is known as **stationary phase** or plateau phase.

When the natural process of cell death predominates, a cell population is considered to be in **death phase**

It is important to note that the amount of time spent in each phase **differs between individual cell lines and cultures**

Anchorage dependent cells

At **confluence**, **growth stops,** and cells must be detached and transferred to new plates.

This action is called **subculturing** or **passaging the cells**.

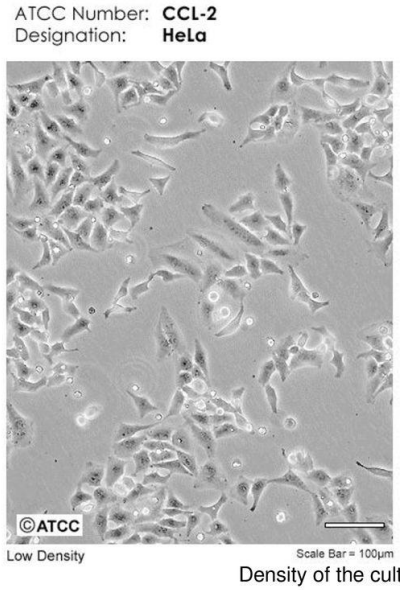
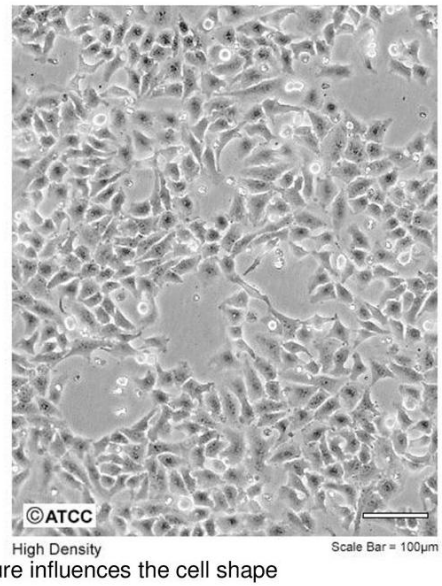
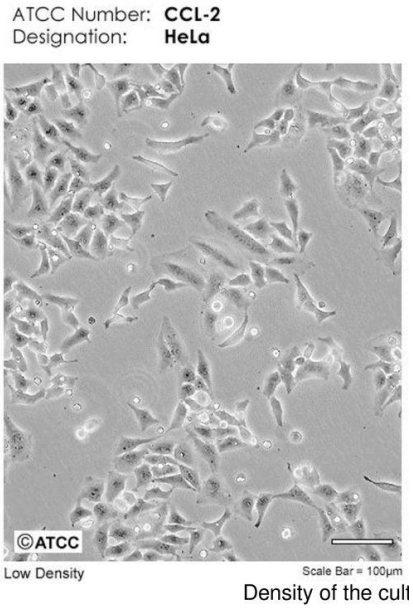


Plate cells at correct seeding density

Growth





Collect and dilute

7 Critical Numbers in Cell Culture Every Researcher Should Know

https://www.atlantisbioscience.com/blog/7-critical-numbers-in-cell-culture-every-researcher-should-know/

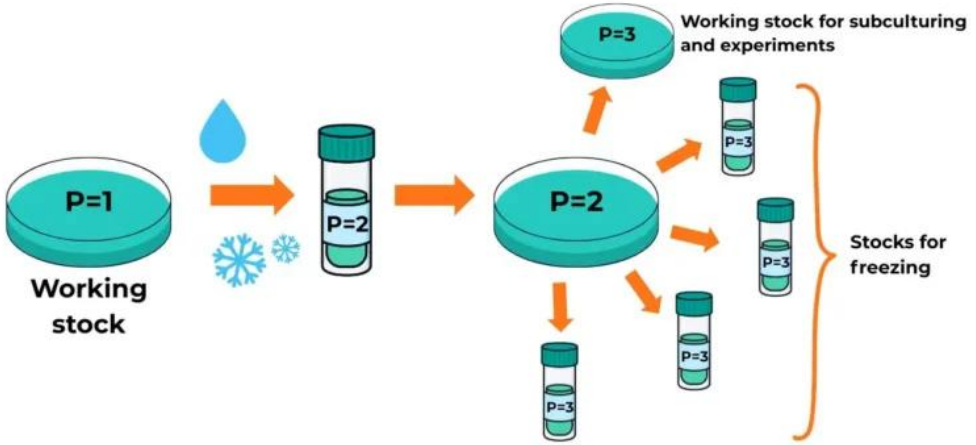
Cell Passaging

Cell passaging or splitting is a **technique** that enables an individual to **keep cells alive and growing** under cultured conditions for extended periods of time

What does 'passage number' mean?

The **passage number** of a cell culture is a record of

- the number of times the culture has been subcultured, i.e. harvested and reseeded into multiple 'daughter' cell culture flasks.

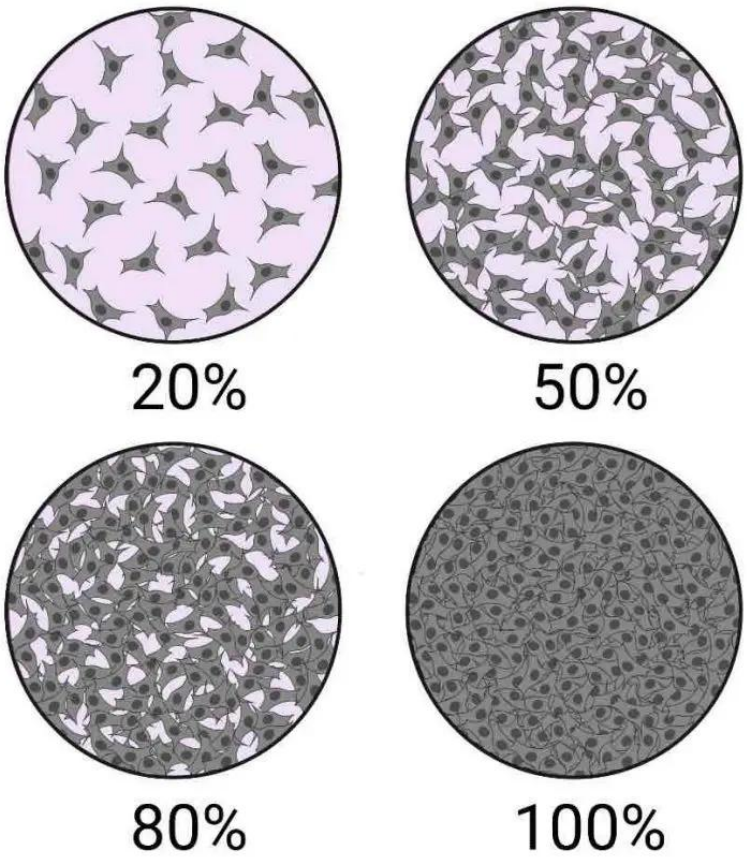


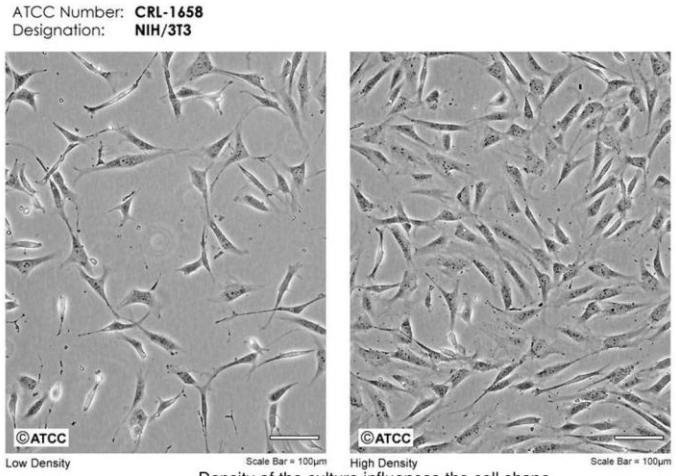
Although passage number is often treated as a routine metric, **it plays a critical role in determining cell quality, performance, and experimental reproducibility**. Over time, cells accumulate molecular and epigenetic changes during in vitro culture. These changes can lead to significant shifts in behaviour, such as altered gene expression, slower proliferation, reduced viability, or unwanted differentiation — even when cell morphology remains unchanged.

Cellular Confluence

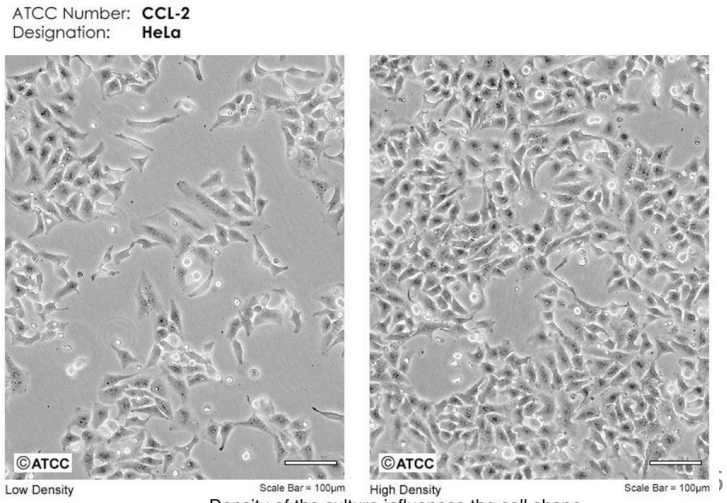
Cellular confluence generally refers to the **percentage of the culture vessel inhabited by attached cells**.

For example, **100% cellular confluence** means the surface area is completely covered by cells, whereas **50% confluence** means roughly half of the surface is covered.





Density of the culture influences the cell shape



Density of the culture influences the cell shape

Cell Seeding Density

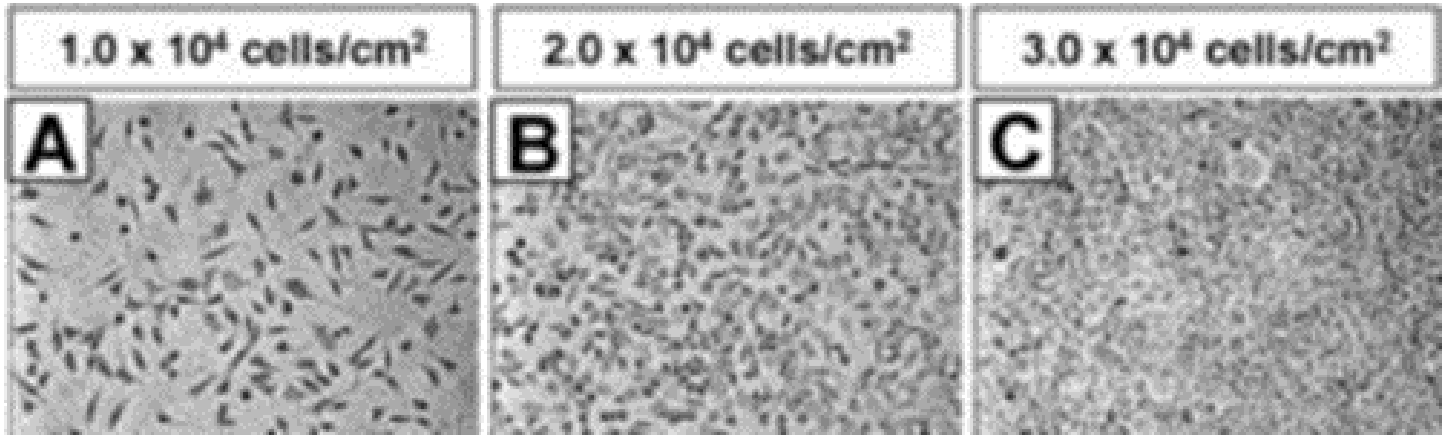
— the number of cells plated per unit area (for adherent cells) or per unit volume (for suspension cells)
— is a **fundamental parameter** in cell culture. It directly influences growth rate, morphology, gene expression, and the outcome of downstream assays.

Incorrect seeding can lead to:

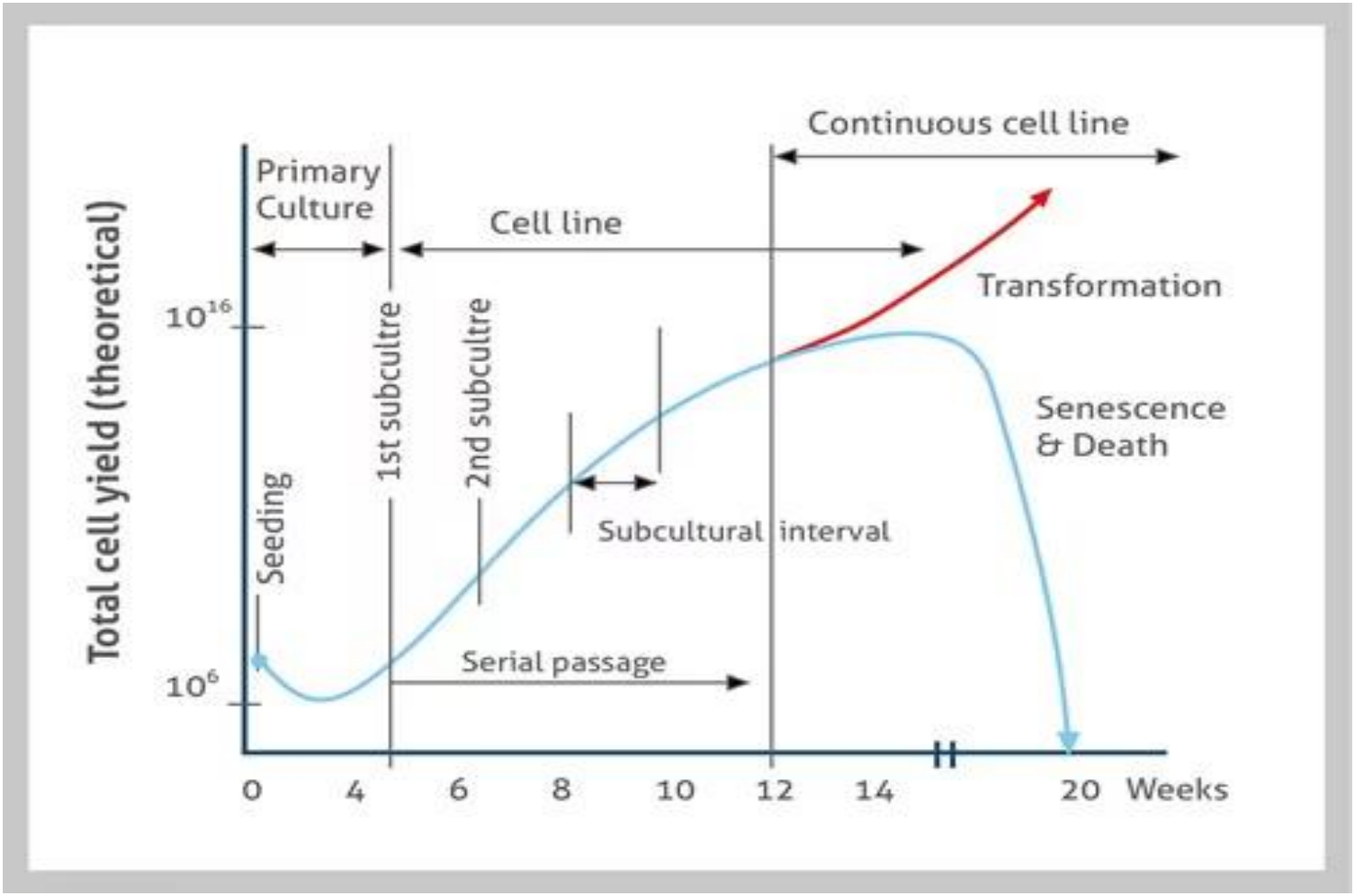
•**Over-confluency,** causing contact inhibition, altered signalling, or early senescence

•**Low density,** which can stress cells, delay growth, or alter behaviour

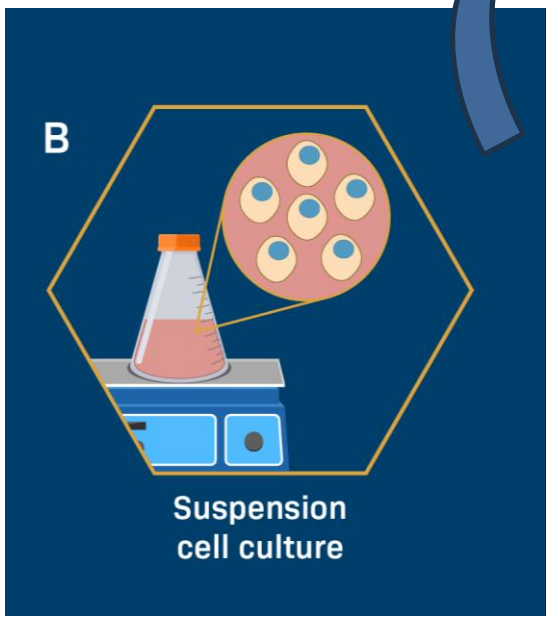
•**Experimental variability,** especially in assays dependent on cell–cell interactions, secreted factors, or differentiation cues



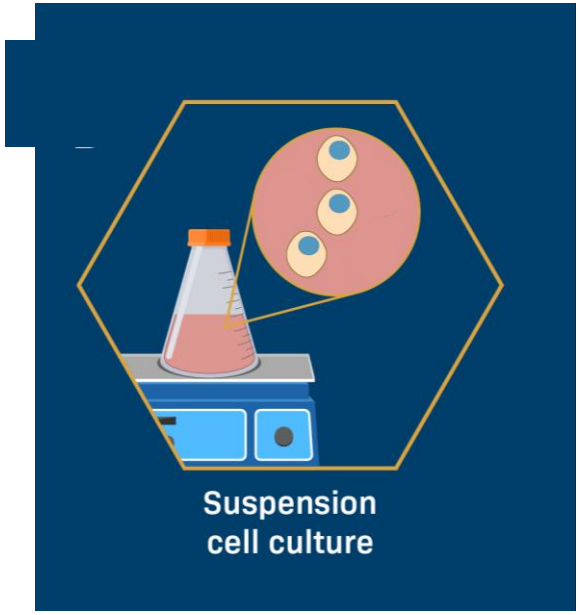
Evolution of a cell culture



Passing Suspension cells



Aseptically remove **1/3rd of medium**



- Easier to passage as **no need to detach them**
- As the suspension cells **reach to confluency**

Replaced with the same amount of pre-warmed medium

Anchorage dependent cells

At **confluence**, **growth stops,** and cells must be detached and transferred to new plates.

This action is called **subculturing** or **passaging the cells**.

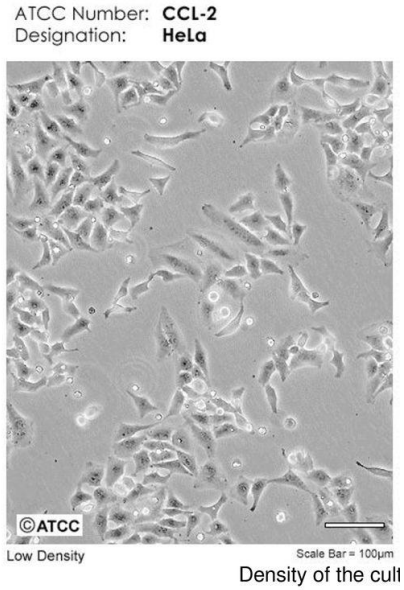
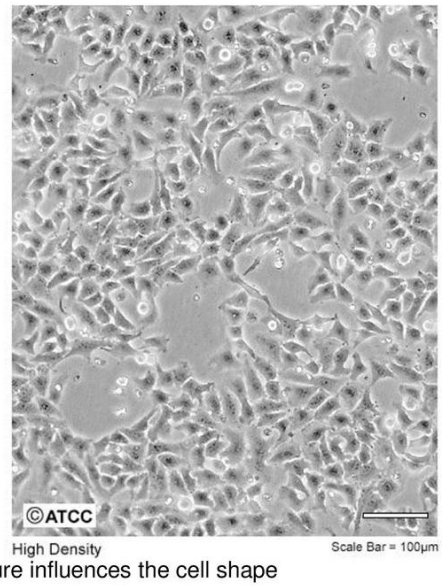
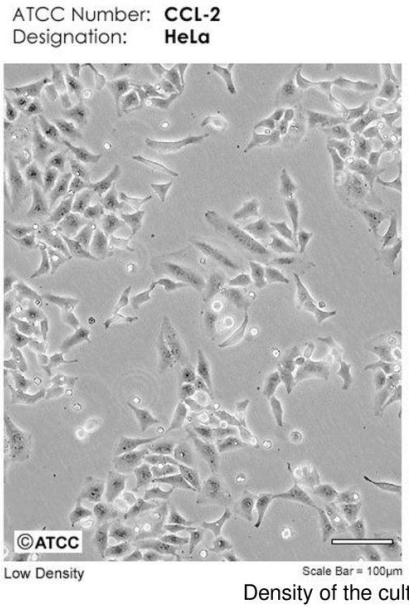


Plate cells at correct seeding density

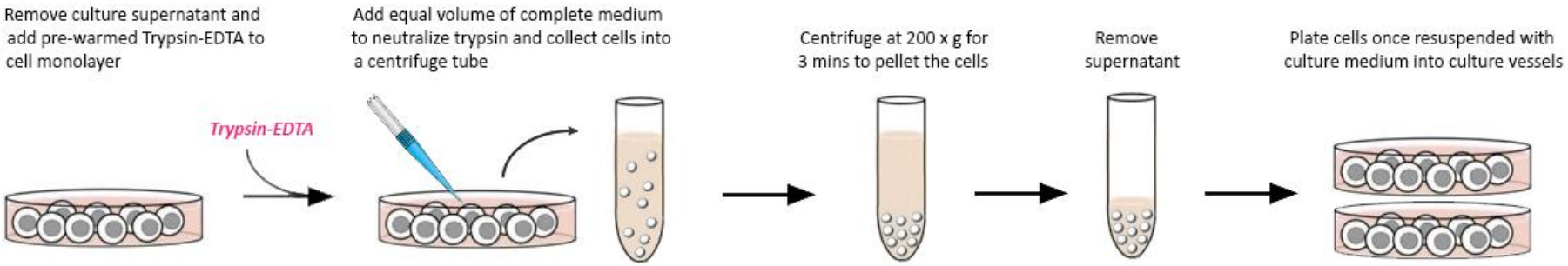
Growth

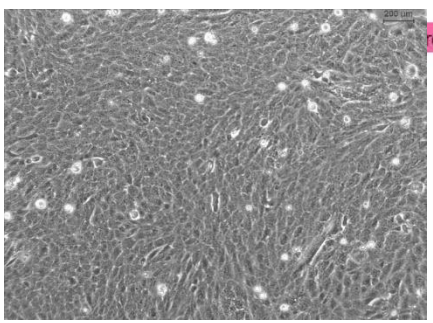


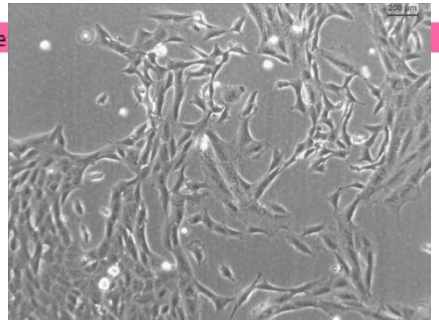


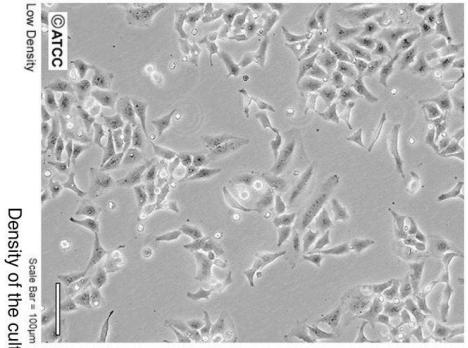
Collect and dilute

Adherent cells



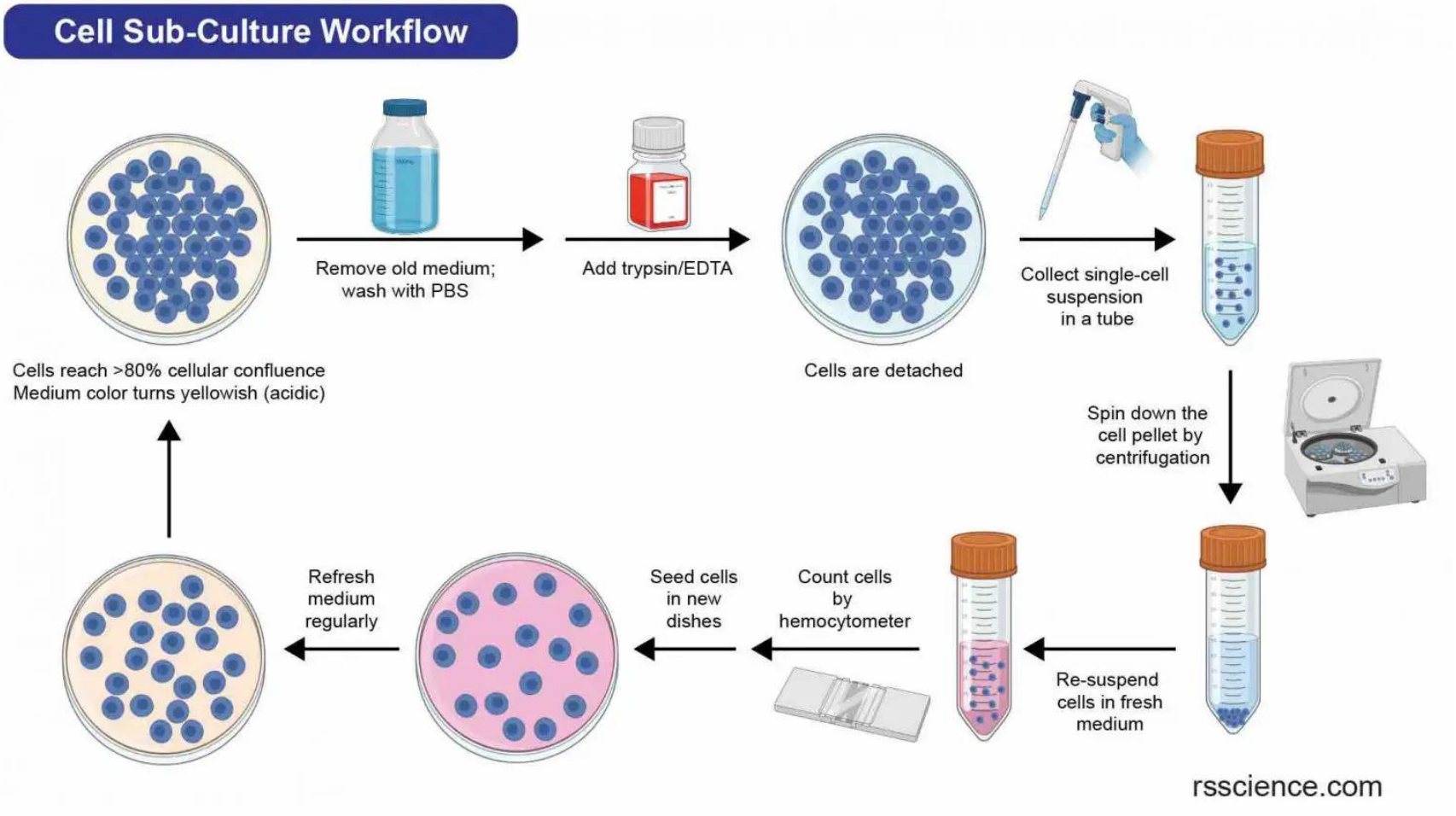






100% confluence

60% confluence



A) For the transfer, the **use of EDTA** (chel Ca^{2+} and Mg^{2+}, indispensable for adhesion) and / or **trypsin** (degrades the proteins of the matrix) are used.

B) After detachment, the action of EDTA and trypsin is neutralized by the addition of a **new culture medium containing excess divalent cations** and trypsin inhibitors.

C) The cells are collected by centrifugation, resuspended, **counted** and **diluted i**n new plates.

Primary cultures

Primary cultures are defined as

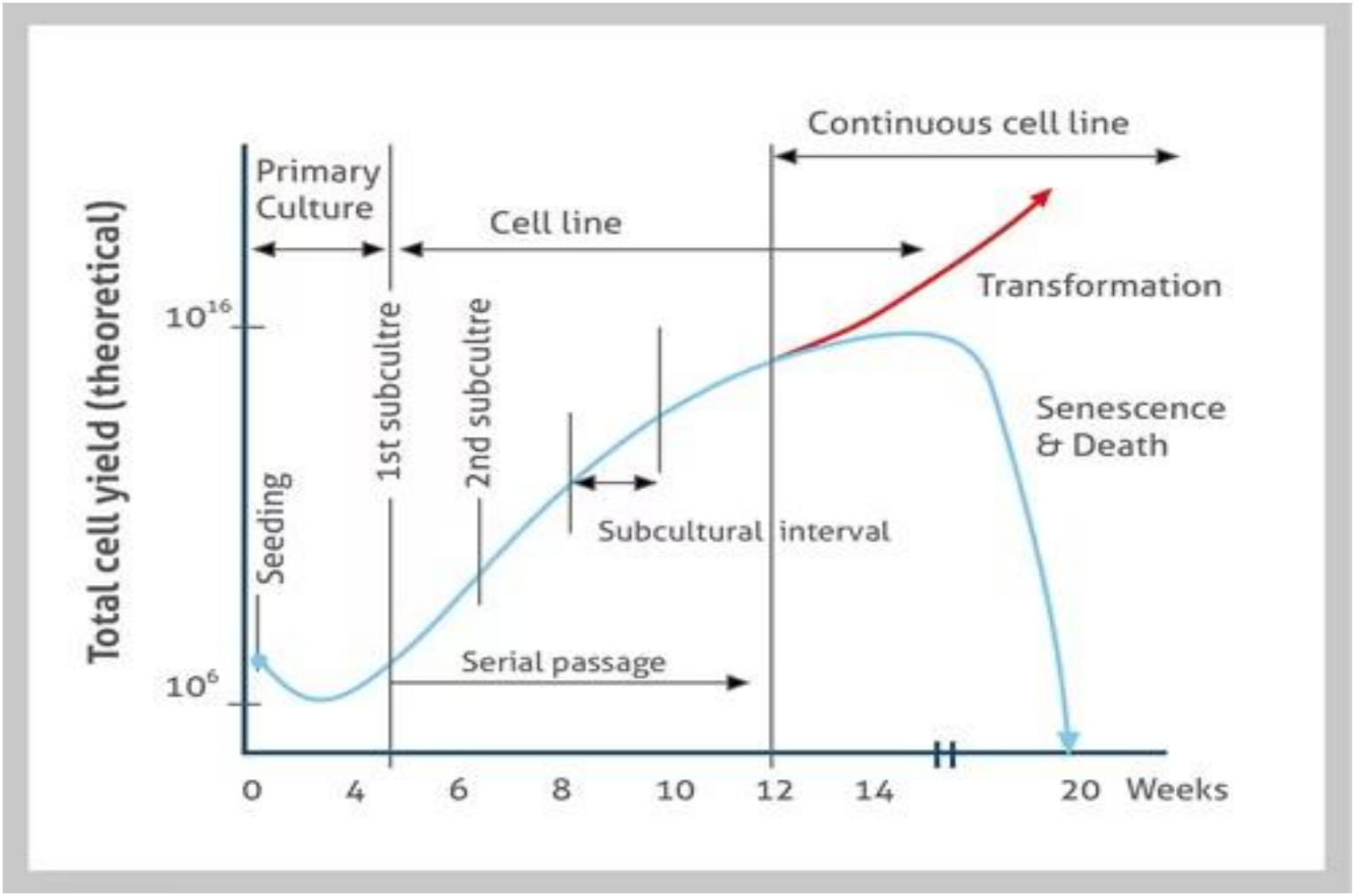
- cultures **obtained from cells coming directly from tissue; embryos, adult** cells in suspension or from tumors.

They generally contain a **relatively heterogeneous mixture of cells in** different physiological states.
There are cells that can grow in suspension or attached to an artificial matrix such as plastic but also to collagen.

Secondary cultures

are subcultures of primary cultures that become necessary when the cells reach confluence or completely cover the matrix to which they are attached.

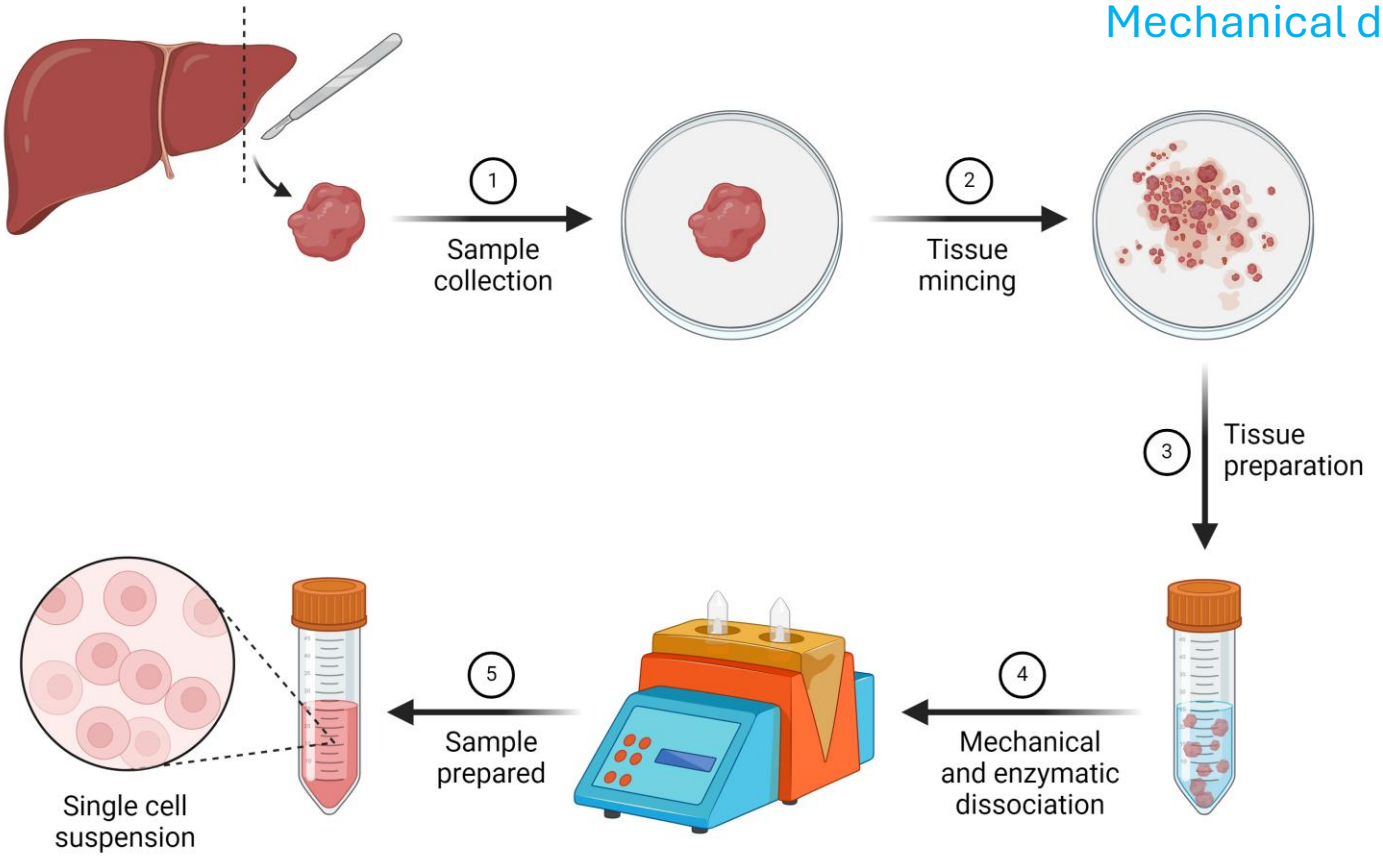
Evolution of a cell culture



Primary cultures

Cells from a sample (organ, biopsy, embryos).

Mechanical dissociation

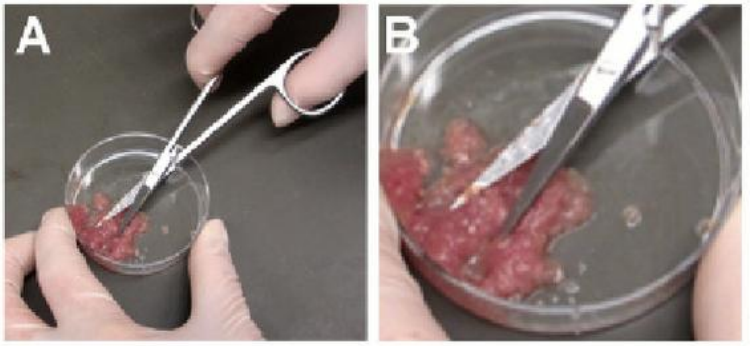


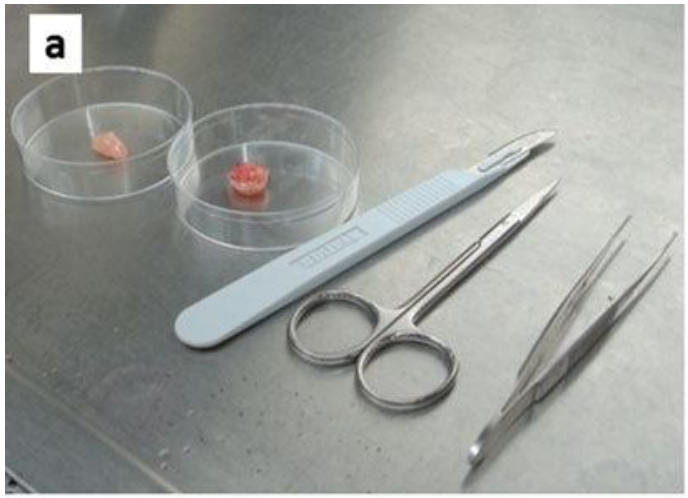
Enzymatic dissociation

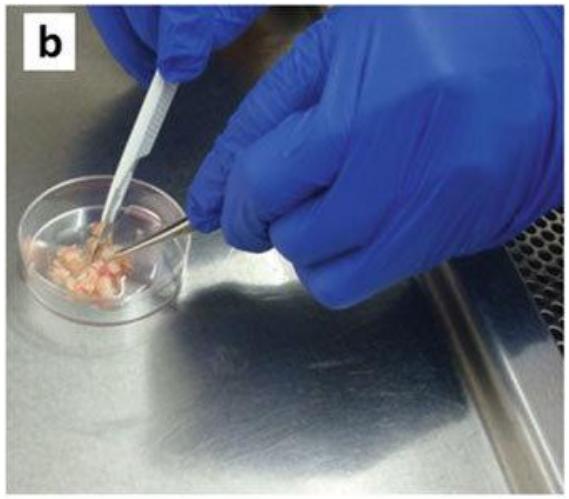
Control of cell viability and plating

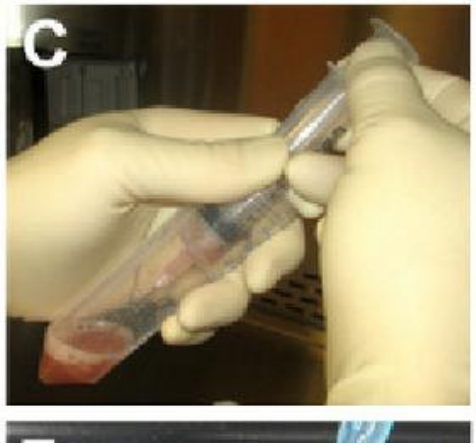
Mechanical dissociation

- repeated mincing with scissors or sharp blades,
- scraping the tissue surface,
- homogenization,
- vortexing, repeated aspiration through pipettes or sequentially smaller needles (e.g., 16-, 20-, and 23-gauge),
- application of abnormal osmolarity stress,









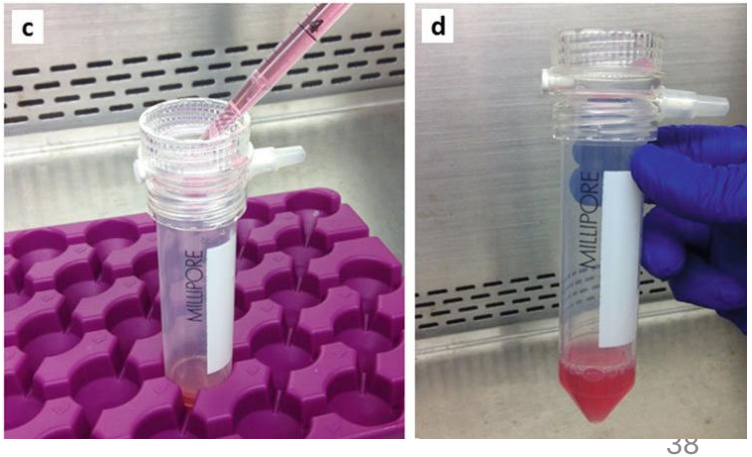
Cell strainers

are sterile, easy to use devices for rapidly isolating primary cells to consistently obtain **a uniform single-cell suspension from tissues**. Fits 50 ml Falcon® conical tubes.

- filtration through a nylon or steel mesh (**50–100 µm opening**),

This process **generates single cell suspensions quickly** with a minimal number of steps.

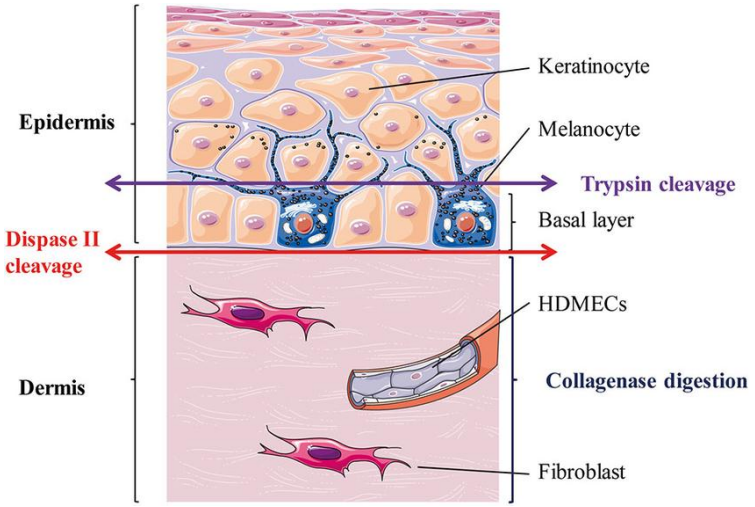
However, dissociation of tumor tissue using mechanical methods is not often a suitable technique, for obtaining tumor cells for culture because the technique results in **a high percentage of dead cells** that secrete degrading enzymes



Enzymatic dissociation

Enzymatic dissociation is a commonly used practice to digest minced tissue into a single cell suspensions due to proper digestion of tissue and preservation of cell viability and integrity.

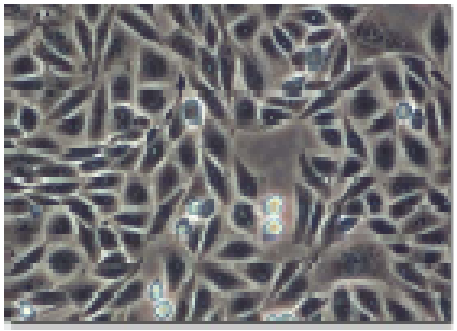
Numerous studies have shown that **certain enzymes are more effective than others** for dissociation of specific tissues.



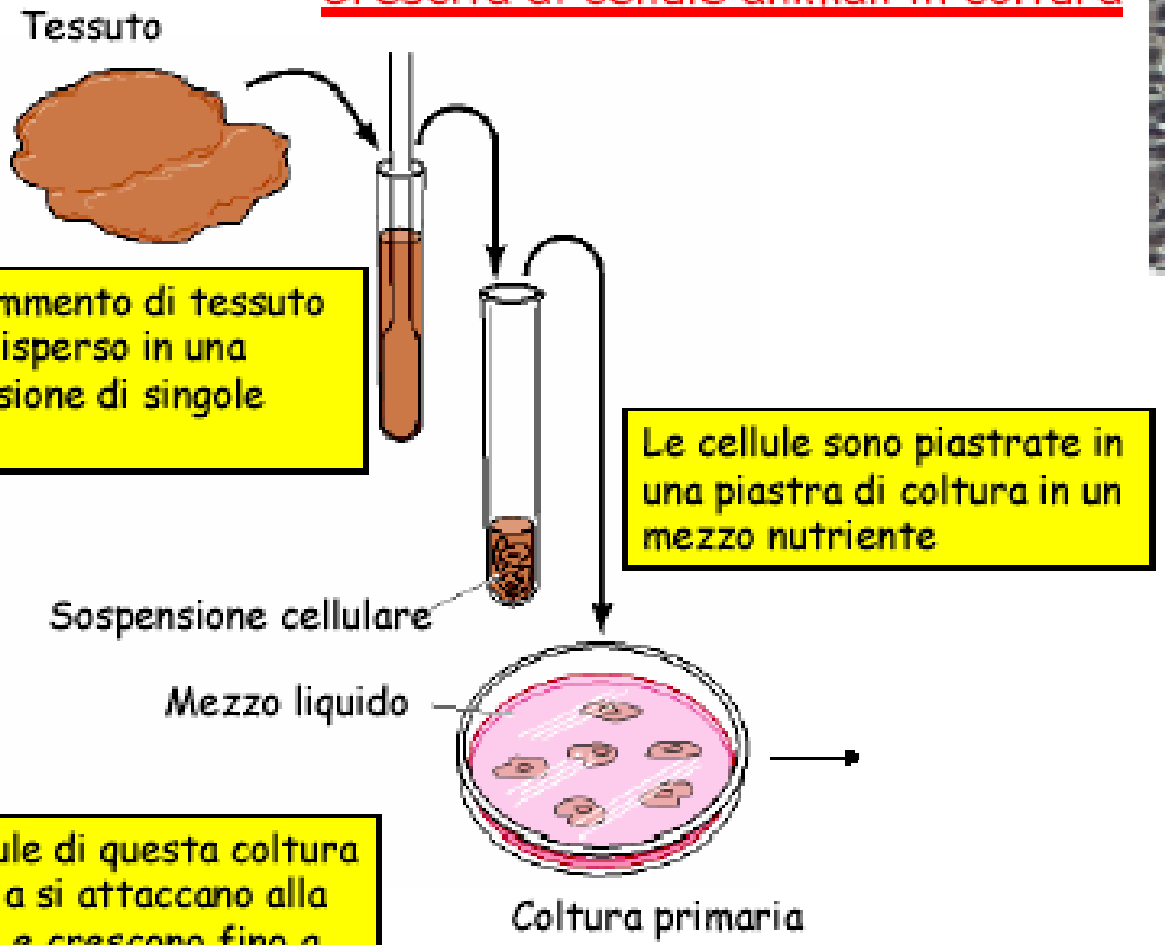
- **Trypsin**

- **papain,**
- **elastase,**
- **hyaluronidase,**
- **collagenase,**
- **pronase,**
- **deoxyribonuclease.**

Crescita di cellule animali in coltura



Cellule di ovaio di criceto



Primary culture

The cells are derived directly from **the original tissue** and retain many functional features that are also found in vivo.

During growth, **selection occurs** based on the degree of proliferation:
- expansion of proliferating cells,
- those that survive but cannot duplicate remain stationary,
- while other cell types are not resistant.

This means that **the proportions of the different cell types change over time** until an equilibrium is reached that is significantly different from the initial situation.

When the proliferation has depleted the culture medium, a subculture must be established in a new container with fresh medium

Types of cell cultured *in vitro*

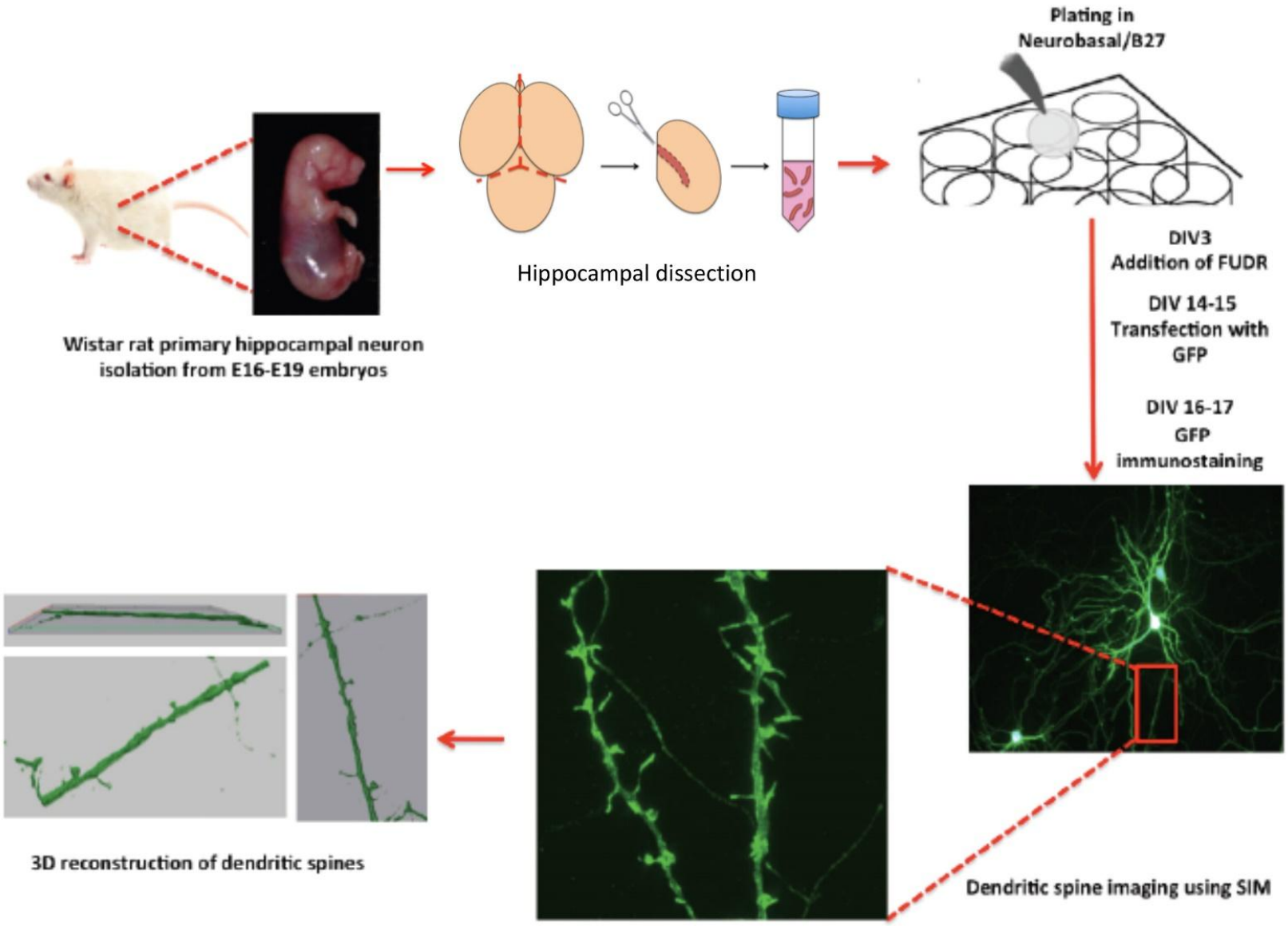
Primary cultures

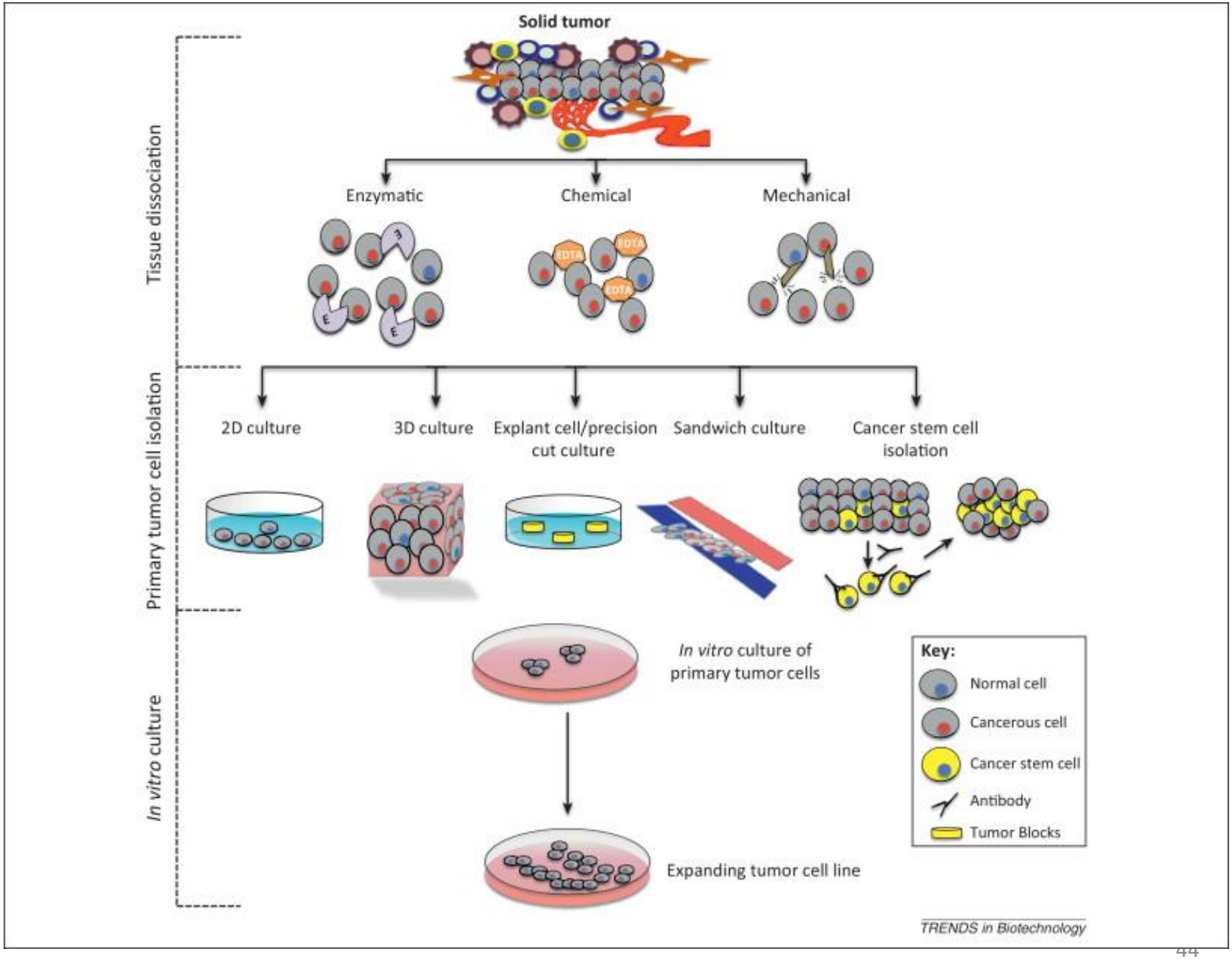
- Derived directly from animal tissue
- embryo or adult?/ Normal or neoplastic?
- Cultured either as tissue explants or single cells
- Initially heterogeneous – become overpopulated with fibroblasts
- **Finite life span** *in vitro*
- Retain **differentiated phenotype**
- Mainly anchorage dependant
- Exhibit contact inhibition

Secondary cultures

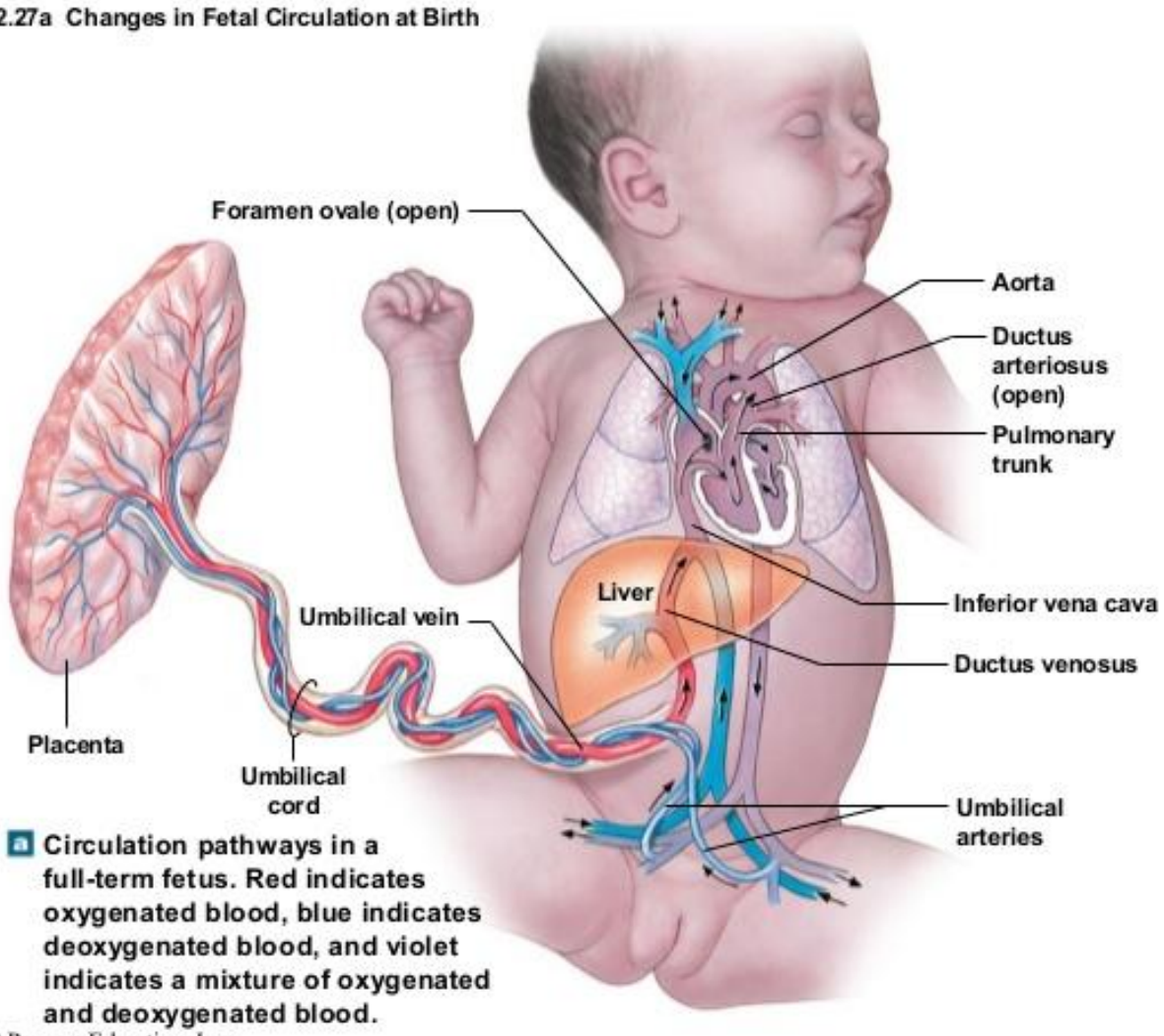
Derived from a primary cell culture

- Isolated by selection or cloning
- Becoming a more homogeneous cell population
- **Finite life span** *in vitro*
- Retain **differentiated phenotype**
- Mainly anchorage dependant
- Exhibit contact inhibition

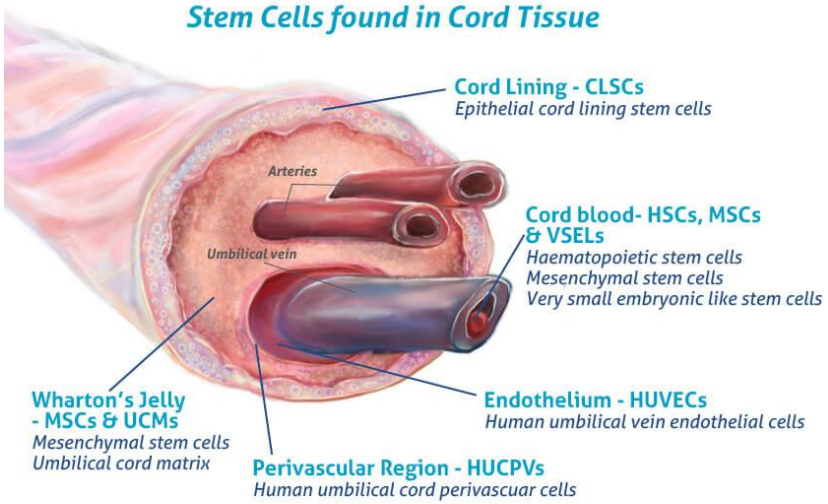

Plating in
Neurobasal/B27
Wistar rat primary hippocampal neuron
isolation from E16-E19 embryos
Hippocampal dissection
DIV3
Addition of FUDR
DIV 14-15
Transfection with
GFP
DIV 16-17
GFP
immunostaining
Dendritic spine imaging using SIM
3D reconstruction of dendritic spines

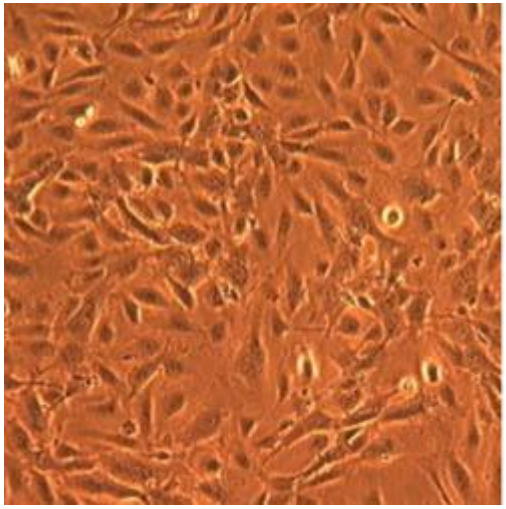

Solid tumor
Tissue dissociation
Enzymatic
Chemical
Mechanical
EDTA
EDTA
EDTA
Primary tumor cell isolation
2D culture
3D culture
Explant cell/precision cut culture
Sandwich culture
Cancer stem cell isolation
In vitro culture
In vitro culture of primary tumor cells
Expanding tumor cell line
Key:
Normal cell
Cancerous cell
Cancer stem cell
Antibody
Tumor Blocks
TRENDS in Biotechnology

HUVEC - Human Umbilical Vein Endothelial Cells



HUVEC Human Umbilical Vein Endothelial Cell





Video isolamento cellule x2

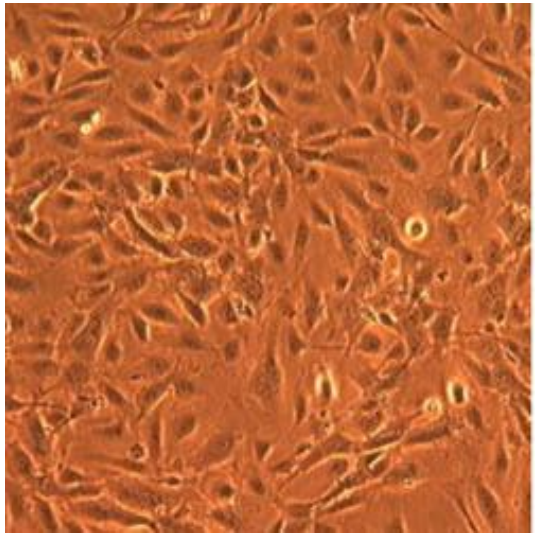
isolation from umbilical cord was first described in 1973.

To date, this model is still widely used because of the **high HUVEC isolation success rate**, and because HUVEC are **an excellent model to study a broad array of diseases**, including cardiovascular and metabolic diseases.

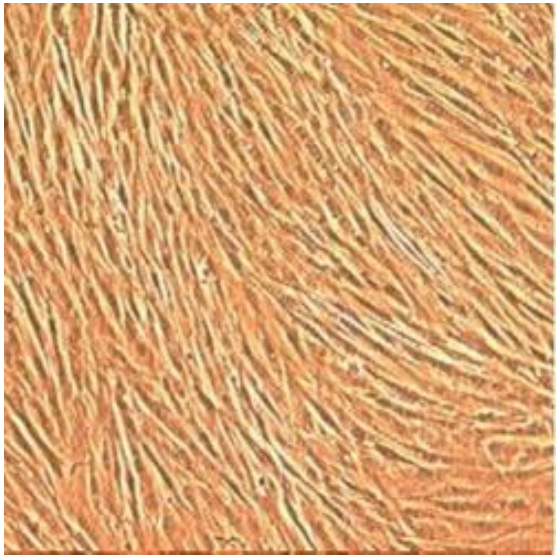
https://www.stemcell.com/how-to-prepare-a-single-cell-suspension-from-mouse-spleen.html

https://www.youtube.com/watch?v=gePfThd2Hro

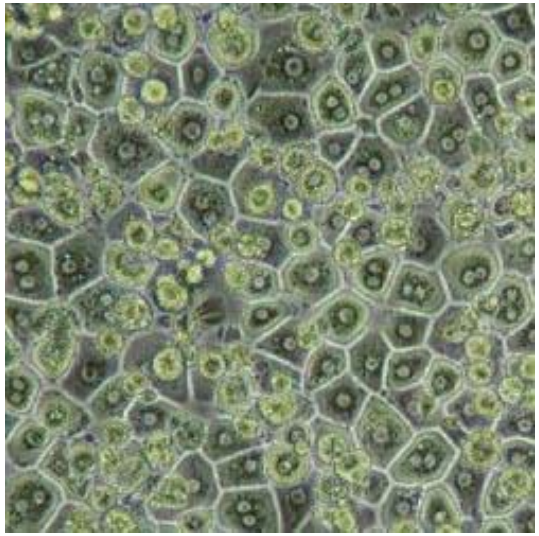
HUVEC
Human Umbilical Vein Endothelial Cells



NHLF
Normal Human Lung Fibroblasts



Human **Hepatocytes**



Normal Human Epidermal **Keratinocytes**