Mammalian cell culture

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What Is Mammalian Cell Culture?

- Mammalian cell culture refers to the growing of animal or human cells outside the body under controlled laboratory conditions.
- The cells are grown in sterile plastic flasks, dishes, or multi-well plates.
- Unlike bacteria, mammalian cells need a more carefully controlled environment.
- They require:
  - suitable culture medium
  - correct temperature
  - controlled CO_2
  - humidity
  - sterile handling
  - regular passaging
  - careful monitoring under the microscope

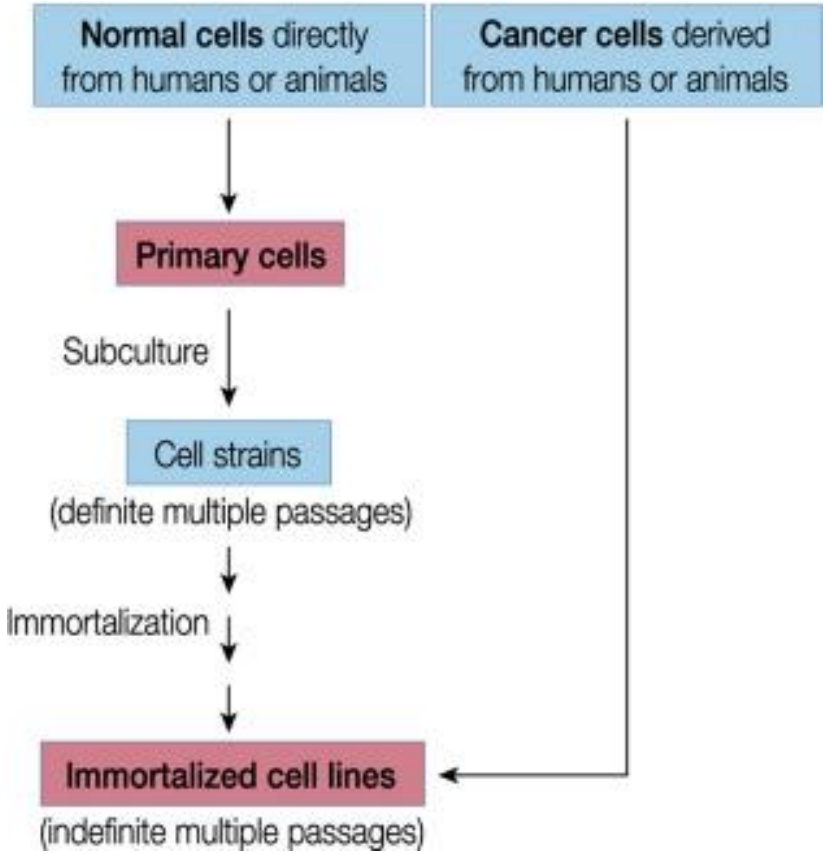
Why Mammalian Cells Are Used in Research

- Mammalian cells are harder and more expensive to grow than bacteria or yeast.
- They are still used because they can perform biological processes that simpler organisms cannot reproduce properly.
- Mammalian cells are useful for studying:
  - cell signalling
  - cancer biology
  - immune responses
  - drug effects
  - toxicity
  - infection
  - protein secretion
  - receptor activity
- They are also important for protein expression.
- Some proteins need correct folding, glycosylation,

  disulphide bond formation, secretion, or processing.
- Bacteria cannot do these properly.

Primary Cells, Cell Lines, and Stem Cells

- There are different types of mammalian cells used in culture.
- **Primary cells** are taken directly from tissue.
  - They are often closer to real biology, but they usually grow for only a limited number of passages.
  - They can be difficult to maintain and may vary between donors.
- **Cell lines** come from cells that can keep dividing for a long time.
  - Many come from tumours or have been transformed by viral or genetic changes.
  - They are easier to grow, but they may not behave like normal tissue.
- **Stem cells** can self-renew and differentiate, but they need tightly controlled culture conditions.
  - The choice depends on the experiment.
  - A convenient cell line is not always the most biologically realistic model.

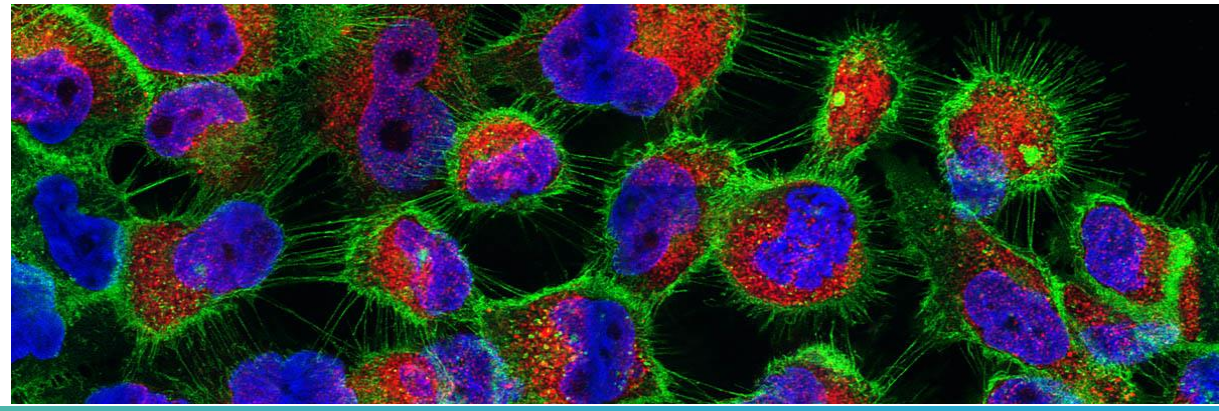


Mammalian Cell Lines

Widely used mammalian cell lines include:

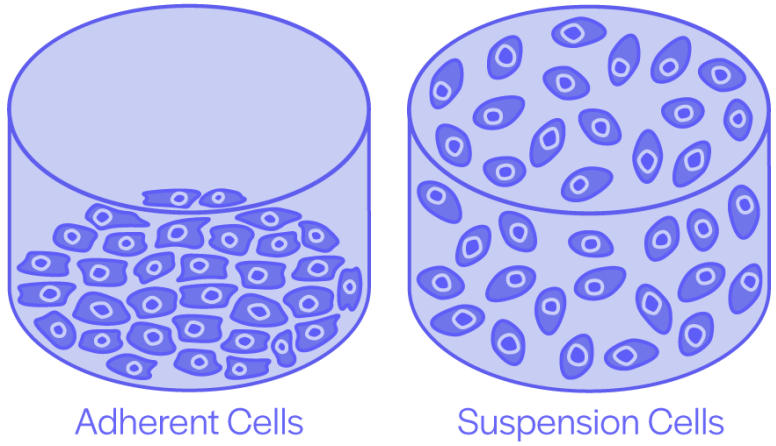
- **HEK-293** - often used for transfection and protein expression
- **HeLa** - robust human cancer-derived cells
- **CHO** - widely used in biotechnology and recombinant protein production
- **Jurkat** - T-cell leukaemia line used in immunology
- **THP-1** - monocyte-like cells often used in inflammation studies
- **HepG2** - liver cancer-derived cells used in metabolism and toxicity work

- Each cell line has its own behaviour.
- They differ in:
  - growth rate
  - shape
  - preferred medium
  - attachment strength
  - transfection efficiency
  - sensitivity to stress



Adherent vs Suspension Cell Culture

- Mammalian cells usually grow in one of two ways:
- **Adherent cells** attach to the plastic surface.
  - Eg: HeLa, HepG2, L929, and many HEK-293 strains
  - These cells are passaged by detaching them from the flask surface.
  - This is usually done using trypsin-EDTA.
- **Suspension cells** grow floating in the medium.
  - Eg: Jurkat, THP-1, and some adapted HEK-293 cells
  - These are usually passaged by counting and diluting them into fresh medium
  - Adherent cells are managed by surface coverage.
  - Suspension cells are managed by cell concentration.



The Cell Culture Environment

- cells are kept close to body-like conditions and are grown in a CO_2 incubator.
- These are the main conditions:
  - 37°C
  - 5% CO_2
  - humidified atmosphere
  - sterile culture vessels
  - nutrient-rich medium
  - pH range 7.2 - 7.4
  - enough space to grow
- The CO_2 helps maintain the pH of bicarbonate-buffered medium.
- Phenol red in the medium gives a useful colour guide:
  - red-orange: usually acceptable
  - yellow: acidic
  - purple-pink: alkaline
- The water tray helps prevent evaporation.
- This matters especially for plates, where small volumes dry out quickly.
- Do not leave the incubator door open longer than needed.
- Every opening disturbs temperature, CO_2, and humidity.

Culture Media

- Unlike bacterial cell cultures, mammalian cells need complex liquid media containing salts, glucose, amino acids, vitamins, buffers, and other nutrients
- Common media include:
  - DMEM
  - RPMI-1640
  - MEM / EMEM
- RPMI is often used for immune-type cells, such as Jurkat or THP-1
- DMEM is commonly used for many adherent cell lines

DMEM, RPMI, Serum, and Supplements

- Culture medium is usually supplemented before use.
- A typical complete medium may contain:
  - basal medium such as DMEM or RPMI
  - 10% foetal bovine serum / foetal calf serum
  - 1% penicillin-streptomycin
  - Sometimes L-glutamine
- Serum provides growth factors, attachment factors, hormones, and proteins that help cells survive.
- L-glutamine supports metabolism, but it can break down over time.
- Some media already contain stable glutamine substitutes.
- A common complete medium is:
  - DMEM + 10% FBS + 1% Pen/Strep, or
  - RPMI + 10% FBS + 1% Pen/Strep

Antibiotics

- Antibiotics are often added to culture medium.
- These reduce the risk of some bacterial contamination.
- But antibiotics do not solve poor technique.
- They do not reliably prevent:
  - fungal contamination
  - yeast contamination
  - mycoplasma
  - contamination from dirty water baths
  - contamination from touching sterile tips
  - contamination from leaving bottles open
- Good aseptic technique is more important than relying on antibiotics.

Culture Vessels

- Mammalian cells are usually grown in sterile plastic vessels.
- Common used vessels include:
  - T25 flasks
  - T75 flasks
  - T175 flasks
  - 6-well plates
  - 12-well plates
  - 24-well plates
  - 96-well plates
- The “T” number refers to growth surface area.
- For example: T25 = 25 cm^2
- Adherent cells need treated plastic so they can attach.
- Flasks often have filter caps to allow gas exchange while reducing contamination risk.
- Do not wet the cap.
- Liquid around the cap can carry microbes into the flask.

Aseptic Technique: The Core Skill in Cell Culture

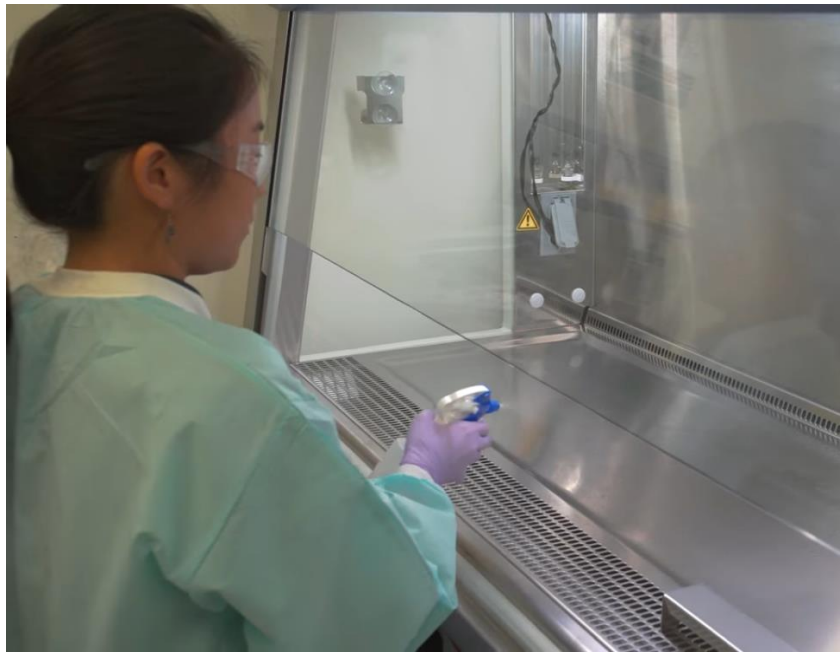
- Aseptic technique is the main practical skill in cell culture.
- The aim is to stop microbes entering sterile media, flasks, plates, and cell suspensions.
- Common contamination sources include:
  - hands
  - sleeves
  - dust
  - bottle caps
  - pipette tips
  - water baths
  - shared incubators
  - crowded cabinets
- The rule is simple:
  - sterile liquids and sterile openings must not contact non-sterile surfaces.
  - Avoid reaching over open vessels.
  - Avoid leaving bottles open.
  - Avoid touching pipette tips on anything except the sterile liquid or vessel they are meant for.
- Most contamination comes from handling, not from the cells themselves.

The Class II Safety Cabinet

- Mammalian cell culture is usually done in a Class II biological safety cabinet.
- The cabinet protects:
  - the culture
  - the worker
  - the room
- Filtered air flows down over the work area.
- There is also an air curtain at the front opening.
- This airflow is important.
- It can be disrupted by:
  - rapid arm movement
  - blocking the front grille
  - too many items in the cabinet
  - placing bottles too close together
  - working too near the front edge
- The cabinet is not magic.
- It only works if the airflow is respected.

Preparing the Hood Before Starting Work

- Before starting, prepare the cabinet properly.
- A typical routine is:
  - turn on the cabinet and allow airflow to stabilise
  - spray the work surface with 70% ethanol or 70% IPA/IMS
  - wipe the surface clean
  - spray bottles and tubes before placing them inside
  - dry bottles before opening them
  - bring in only what is needed
  - keep waste containers within reach
  - Do not overcrowd the hood.
- Too many items make it harder to work cleanly.
- The outside of bottles and tubes is not sterile.
- Spraying reduces risk, but it does not make careless handling safe.



Working Cleanly

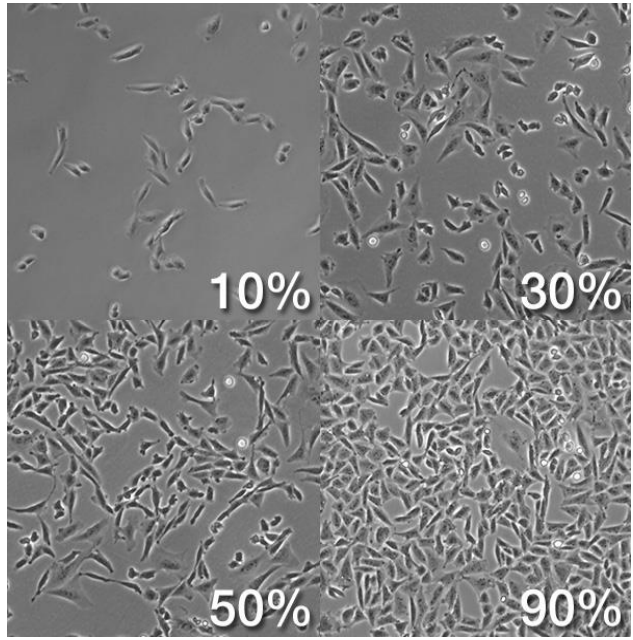
- Good cell culture depends on small habits.
- Important habits include:
  - keep hands away from open vessels
  - do not pass sleeves over open plates or bottles
  - keep bottle caps controlled
  - close bottles as soon as possible
  - avoid touching sterile tips on surfaces
  - discard any tip if you are unsure
  - keep clean items away from waste
  - work steadily, not quickly
- A pipette tip is cheap.
- A contaminated culture is expensive.
- If a tip touches the hood surface, your glove, the outside of a bottle, or the edge of a waste container, discard it.
- Do not try to rescue doubtful sterile technique.

Cell Morphology and Daily Inspection

- Cells should be checked regularly under an inverted microscope.
- This should be done before passaging or setting up experiments.
- Look for:
  - expected cell shape
  - even attachment
  - normal growth rate
  - suitable confluence
  - floating dead cells
  - medium colour change
  - visible contamination
- Healthy adherent cells are often spread out and attached.
- Unhealthy cells may look:
  - rounded
  - shiny
  - detached
  - granular
  - clumped
  - sparse
  - slow-growing

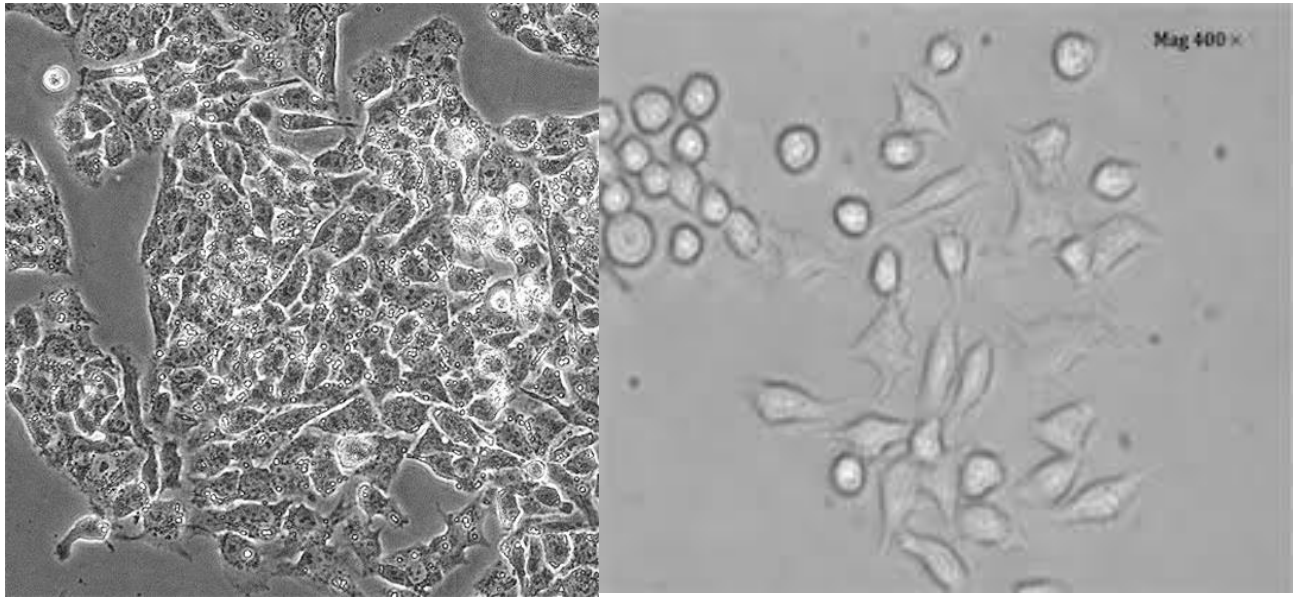
Confluence: Knowing When Cells Are Ready

- Confluence means how much of the flask or well surface is covered by adherent cells.
- Examples:
  - 30% confluent: cells cover about one-third of the surface
  - 70% confluent: cells cover most of the surface but still have space
  - 100% confluent: the surface is fully covered
- Adherent cells are split at around 70–85% confluence.
- They should usually not be left beyond 90%.
- Over-confluent cells may:
  - stop growing well
  - change shape
  - detach
  - become stressed
  - alter gene expression
  - respond differently to treatments
- Confluence affects biology.
- It is not just a visual estimate.



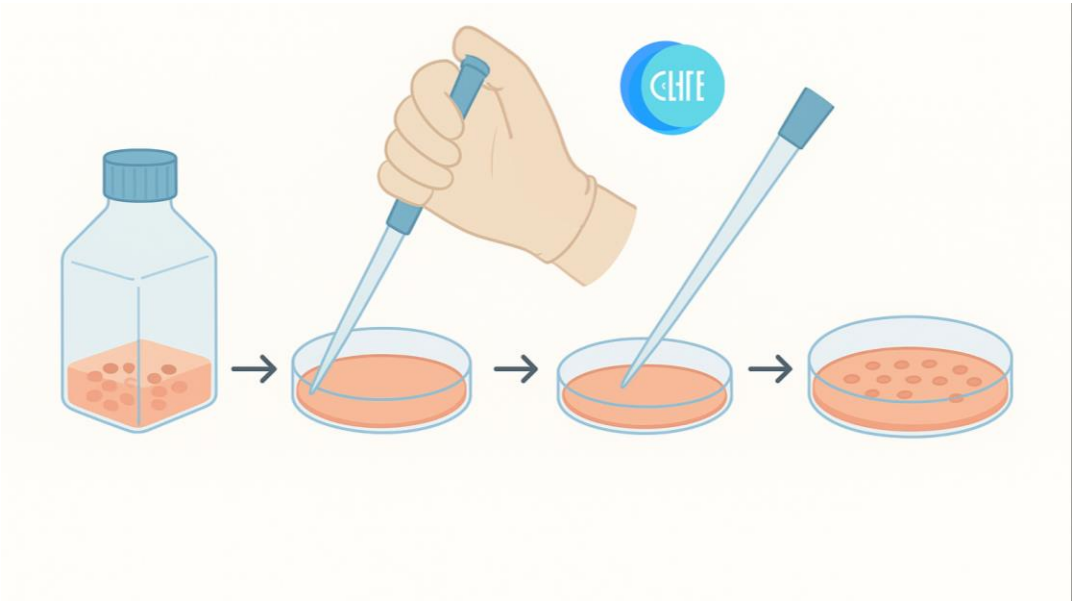
Subculturing: Keeping Cells in Log-Phase Growth

- Subculturing means moving cells into fresh medium and a new vessel.
- It is also called:
  - passaging
  - splitting
  - replating
- Cells are subcultured because they keep dividing.
- If they are left too long, they become crowded, stressed, and less useful experimentally.
- The aim is to keep cells in active growth.
- How often cells need splitting depends on:
  - cell line
  - growth rate
  - starting density
  - flask size
  - medium volume
  - experiment timing
- Some fast-growing cells need splitting every 2–3 days.
- Slower cells may need longer.



Passaging Adherent Cells

- Adherent cells must be detached from the plastic before splitting.
- A typical workflow is:
  - check cells under the microscope
  - remove old medium
  - wash with sterile PBS
  - add trypsin-EDTA
  - incubate briefly at 37°C
  - check that cells have rounded up
  - tap the flask gently if needed
  - neutralise trypsin with serum-containing medium
  - collect the cells
  - centrifuge if required
  - resuspend in fresh medium
  - seed into a new flask or plate
- Do not leave cells in trypsin longer than necessary.
- If cells are difficult to detach, check the cell line protocol rather than simply increasing trypsin time.



Trypsin-EDTA and Cell Detachment

- Trypsin-EDTA is used to detach adherent cells.
- It works because:
  - trypsin digests attachment proteins
  - EDTA disrupts calcium- and magnesium-dependent adhesion
- After trypsin is added, cells usually:
  - round up
  - lose their spread shape
  - detach from the surface
  - float into suspension
- Typical exposure is often around 2–5 minutes, depending on the cell line.

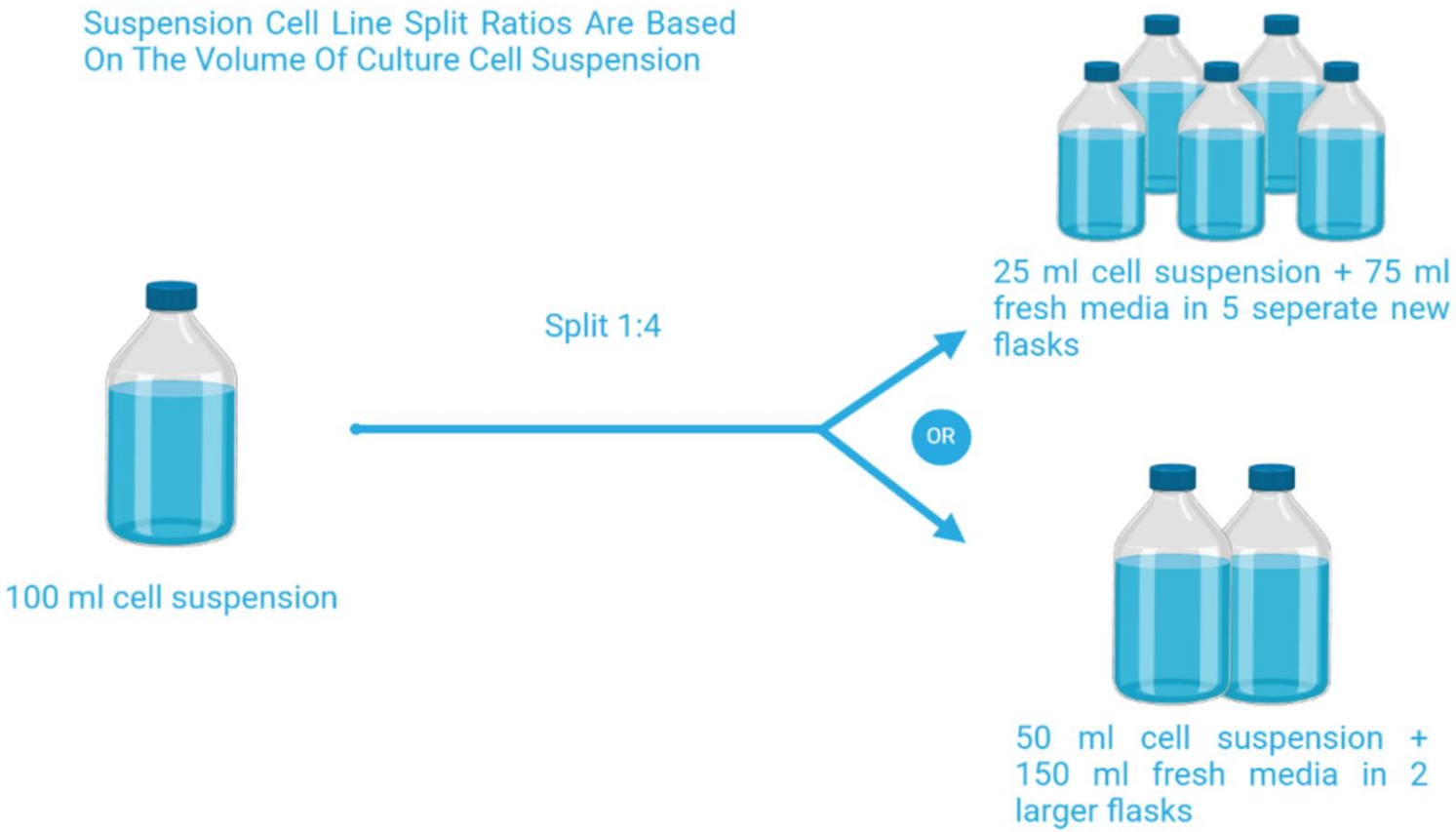
Split Ratios and Seeding Density

- A split ratio describes how much of the cell suspension is transferred into a new flask.
- Examples:
  - 1:2 split: half the cells are transferred
  - 1:5 split: one fifth is transferred
  - 1:10 split: one tenth is transferred
- Many robust cell lines are split somewhere between 1:3 and 1:10.

Passaging Suspension Cells

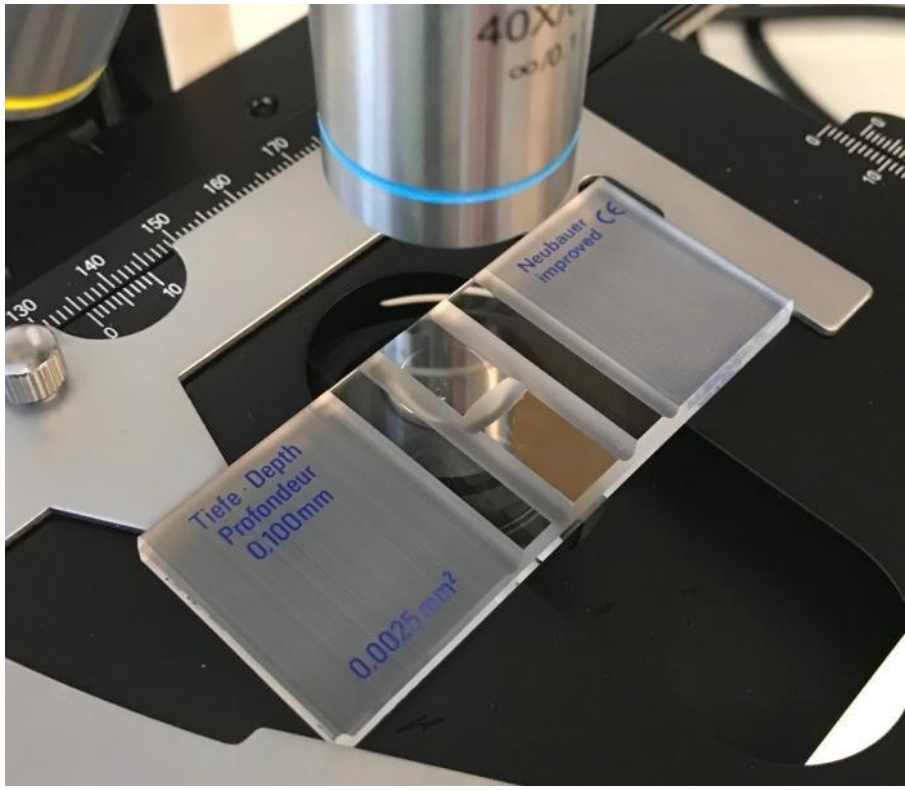
- Suspension cells are easier to passage because they do not need detachment.
- A typical workflow is:
  - mix the flask gently
  - take a sample
  - count the cells
  - calculate the current concentration
  - remove the required volume
  - dilute into fresh medium
  - return cells to the correct density
- Suspension cultures are controlled by

  cell concentration rather than confluence.
- If the culture becomes too dense, cells may:
  - use up nutrients
  - acidify the medium
  - accumulate waste
  - become stressed
  - lose viability

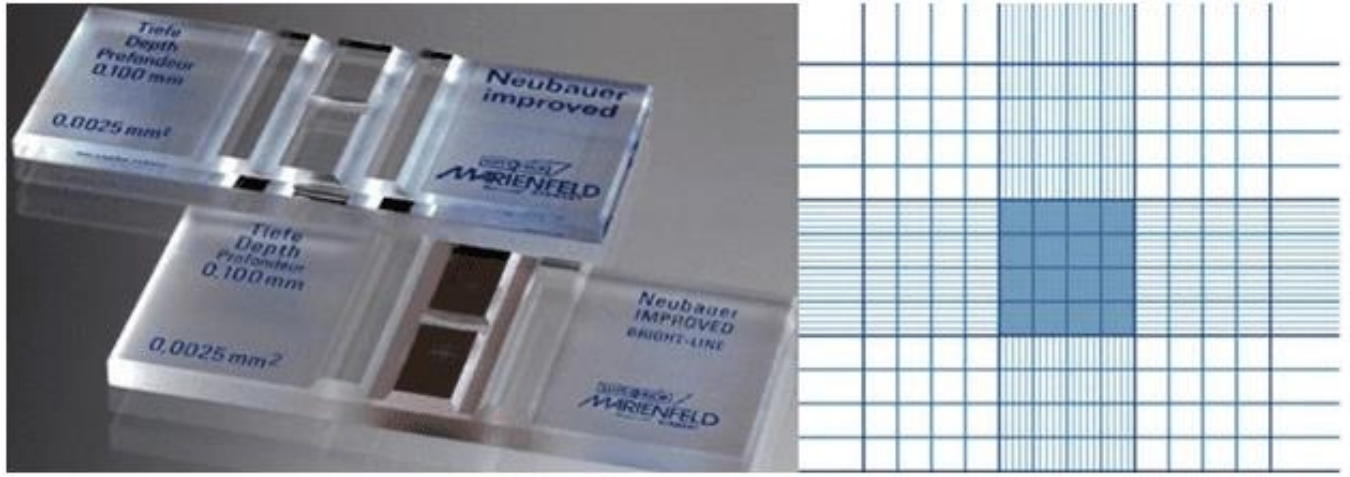


Cell Counting with a Haemocytometer

- A haemocytometer is used to estimate cell concentration.
  - Gently mix the cell suspension
  - load a small volume under the coverslip
  - let the cells settle briefly
  - count cells in defined grid squares
  - calculate cells per ml
- For many standard haemocytometers:
  - cells/ml = average count per large square × dilution factor × 10^4
  - Counting must be done from a well-mixed suspension.
  - If cells settle, clump, or are not mixed properly, the count will be wrong.
  - Bad counting leads to bad seeding.
  - Bad seeding leads to unreliable experiments.



Trypan Blue and Cell Viability



- Trypan blue is used to estimate cell viability.
- The principle is:
  - live cells exclude the dye
  - dead cells take up the dye and appear blue
- A common method is to mix:
  - one volume cell suspension
  - one volume trypan blue
  - This gives a 1:2 dilution.
- That dilution must be included in the cell concentration calculation.
- Viability is calculated as:
  - viability (%) = live cells ÷ total cells × 100
  - For many experiments, viability should ideally be above 85-90%.
- Low viability means the cells may already be stressed before the experiment starts.

Calculating Cell Concentration for Plating

- The standard equation is:
- $C_1V_1 = C_2V_2$
- Where:
  - C_1 = stock concentration
  - V_1 = volume of stock needed
  - C_2 = desired concentration
  - V_2 = final volume needed
- Example:
  - Stock concentration = 4×10^6 cells/ml
  - Desired concentration = 2×10^5 cells/ml
  - Final volume = 25 ml
  - So:
  - $V_1 = C_2V_2 \div C_1$
  - $V_1 = 2 \times 10^5 \times 25 \div 4 \times 10^6$
  - V_1 = 1.25 ml
- So mix:
  - 1.25 ml cell suspension
  - 23.75 ml fresh medium

Setting Up Multi-Well Plate Experiments

- Multi-well plates allow different treatments or conditions to be tested together.
- Common formats include:
  - 6-well plates
  - 12-well plates
  - 24-well plates
  - 96-well plates
- Typical working volumes include:
  - 96-well plate: about 100 µl per well
  - 24-well plate: about 500 µl to 1 ml per well
  - 12-well plate: about 1 ml per well
  - 6-well plate: about 2–3 ml per well
- Always prepare extra cell suspension.
- For example, two 96-well plates need 19.2 ml exactly at 100 µl per well.
- In practice, prepare around 22–25 ml to allow for pipetting loss.
- Keep mixing gently while plating so cells do not settle.

Cryopreservation

- Cells are frozen to preserve stocks for future use.
- Freezing protects against:
  - contamination loss
  - genetic drift
  - over-passaging
  - failed experiments
  - accidental loss of cultures
- A common freezing medium is:
- 90% FBS + 10% DMSO
- DMSO protects cells during freezing, but it is toxic if cells sit in it too long at room temperature.
- Cells should usually be frozen while healthy and actively growing.
- Controlled cooling is important.
- A common target is about −1°C per minute before transfer to long-term storage.

Thawing Cells Without Killing Them

- Thawing should be quick and controlled.
  - remove vial from liquid nitrogen or freezer storage
  - thaw rapidly in a 37°C water bath
  - stop when only a small ice crystal remains
  - spray the vial before taking it into the hood
  - transfer cells into warm complete medium
  - remove or dilute DMSO quickly
  - seed into an appropriate flask
  - change medium after recovery if needed
  - Do not leave cells sitting in DMSO.
  - Do not thaw slowly on the bench.
- After thawing, cells may look stressed for the first day.

Summary

- Good mammalian cell culture depends on consistency.
- The main principles are:
  - use the correct cell type
  - use the correct medium
  - keep cells sterile
  - maintain 37°C and 5% CO_2
  - avoid over-confluence
  - passage cells at the right time
  - count cells properly
  - seed cells evenly
  - check morphology regularly
  - test for mycoplasma
  - record passage number and handling details
  - use proper experimental controls