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Faculty of Natural and Life Sciences

Department of Biological Sciences

Course handout:

Cell culture

Designed for Master's Year 1 students

Specialty: Applied Biochemistry

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Preface

This course handout, titled *Cell Culture with MCQs and Exercises for M1 Applied Biochemistry Students*, is authored by **Dr. MELLALI Sarah**, a Lecturer (Class A) in the Department of Biological Sciences at the Faculty of Science and Technology, and a member of the Environmental and Sustainable Development Laboratory at the University of Relizane, Algeria. This course handout encompasses the course material for *Cell Culture*, including MCQs and exercises, designed for the first year of the Master's program in Applied Biochemistry at the Faculty of Science and Technology, Department of Biological Sciences, at Ahmed Zabana University, Relizane.

This module is an essential and indispensable component of the curriculum for both the first and second years of the Master's program, as well as for students conducting research for their final thesis or even doctoral work. It equips students with the ability to successfully culture cells and apply this technology in their research endeavors.

Prerequisites: To derive maximum benefit from this course, students should possess foundational knowledge in cellular biology, molecular genetics, and basic biochemical laboratory procedures. Familiarity with microbiological concepts and sterile techniques will be advantageous.

The course aims to develop the following competencies in students:

- ✓ The ability to successfully perform cell culture in the laboratory.
- ✓ The ability to manipulate and culture various types of cells based on their origin, research goals, and the nature of their work.
- ✓ The ability to overcome challenges encountered during manipulation and select appropriate methods for their research.

Students will progressively build this complex skill by mastering knowledge, applying practical skills, and adopting a professional attitude.

By the end of this course, students will be able to:

- **In terms of knowledge:**
    - ✓ Learn the terminology and basic concepts of cell culture, facilitating their learning and expanding their knowledge.

- **In terms of practical skills:**

    - ✓ Easily manipulate cultured cells, maintain cultures, and transition from primary to secondary cultures.
    - ✓ Use appropriate protocols for different cell types and research, mastering the preparation of culture media.

- **In terms of professional attitude:**

    - ✓ Respect the requirements of cell culture, such as culture conditions, sterilization, and aseptic techniques.
    - ✓ Overcome challenges encountered during manipulation, such as contamination.

This course details six main areas in cell culture: Introduction to Cell Culture, Infrastructure, Equipment, and Sterilization Techniques, Culture Media: Composition and Preparation; From Isolation to Characterization of Cultured Cells; General Applications of Cell Culture and Specific and Advanced Applications in Cell Culture.

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Chapter 1: Introduction to Cell Culture

1. Definition and History of Cell Culture

Cell culture has emerged as one of the most essential tools in modern life sciences. Cultured cells serve as a critical study material due to their versatility and accessibility. The advantages they provide make them indispensable for both fundamental research and practical applications. Scientists developed cell culture techniques to grow microorganisms outside their native environment under precisely controlled conditions. A wide range of cell types can be cultured, including single-celled microorganisms (e.g., bacteria, yeast) and cells derived from multicellular organisms (plants and animals). By cultivating these cells in laboratory settings, researchers can regulate their growth and produce large quantities of microorganisms or valuable bioactive compounds.
Today, technological breakthroughs play a pivotal role in tackling complex challenges, particularly in refining *in vitro* cell culture methodologies.

1.1. Definition of Cell Culture

Cell culture is an intricate process involving a suite of biological techniques that enable prokaryotic, eukaryotic, or plant cells to proliferate outside their natural habitat (ex vivo) or host organism. This method is employed for scientific experimentation, *in vitro* fertilization, or industrial applications.

At its core, cell culture is a laboratory practice focused on isolating viable cells, ensuring their survival under artificial laboratory conditions, promoting their proliferation, and minimizing cellular stress. The composition and quality of the culture medium are paramount to achieving these goals, as they directly influence cell viability, growth rates, and functionality.

1.2. History of Cell Culture

Scientists have attempted to culture cells in laboratories since the late 19th century, with a series of landmark events shaping its development:

- ✓ **1878:** Claude Bernard proposed that physiological systems could be maintained in a living state even after an organism's death.
- ✓ **1885:** Wilhelm Roux demonstrated that embryonic chicken cells could survive in saline solution outside the animal's body.

- ✓ **1903:** Jolly observed *in vitro* cell division in salamander leukocytes.
- ✓ **1907:** Ross Harrison cultured frog nerve cells in a lymph clot (a semi-solid mass formed by coagulation) and observed nerve fiber growth *in vitro* for weeks, earning him recognition as the "father of cell culture."
- ✓ **1910:** Montrose Burrows achieved long-term culture of embryonic chicken cells in plasma clots and documented mitosis in detail.
- ✓ **1911:** Warren and Margaret Lewis developed the first liquid media (seawater, serum, embryo extract, salts, and peptones) and observed limited monolayer growth.
- ✓ **1912:** French surgeon Alexis Carrel cultured beating chicken heart cells.
- ✓ **1913:** Carrel introduced strict aseptic techniques for prolonged cell cultivation.
- ✓ **1916:** Peyton Rous and Jones pioneered the use of trypsin, a proteolytic enzyme, for subculturing adherent cells.
- ✓ **1923:** Carrel and Baker designed the first dedicated cell culture vessel, the "Carrel flask" (T-flask), enabling microscopic evaluation of cultured cells.
- ✓ **1943:** Wilton Earle isolated mouse L-fibroblasts capable of clonal growth from single cells.
- ✓ **1948:** Penicillin and streptomycin were introduced into culture media, drastically reducing contamination.
- ✓ **1952:** George Gey established the HeLa cell line from a human cervical carcinoma (Henrietta Lacks). Renato Dulbecco developed plaque assays for animal viruses using confluent cell monolayers.
- ✓ **1955:** Harry Eagle formulated the first widely used chemically defined medium, defining cellular nutritional requirements.
- ✓ **1961:** Leonard Hayflick and Paul Moorhead demonstrated the finite lifespan of human fibroblasts (WI-38) in culture.
- ✓ **1965:** Richard Ham created the first serum-free medium. John Harris and Henry Watkins fused human and mouse cells using viruses.
- ✓ **1978:** Hormone and growth factor cocktails laid the groundwork for advanced serum-free media.
- ✓ **1980:** Breakthroughs in gene regulation, cellular morphology, and growth control.
- ✓ **1983:** Cell immortalization achieved via SV40 viral transformation.
- ✓ **1990:** Industrial-scale culture of transfected cells for pharmaceutical production.

- ✓ **2000:** Human Genome Project advancements linked genomics and proteomics to genetic disorders.
- ✓ **2007:** Shinya Yamanaka's team reprogrammed adult cells into induced pluripotent stem cells (iPSCs).
- ✓ **2010:** Japan's AIST and Kawasaki Heavy Industries unveiled the R-CPX robot, enabling co-culture of cells from multiple donors.
- ✓ **2010s–Present:** Three-dimensional (3D) gel-based culture systems revolutionized multicellular modeling and organoid development .

2. Cultured Cells

2.1 Origin of Cultured Cells

Cultured cells derive from diverse biological sources, each requiring specific techniques for isolation and maintenance. The origins are categorized as follows:

A. **Free Microorganisms**
   - ✓ **Examples**:
     - *Bacteria* (e.g., *Escherichia coli* for recombinant protein production).
     - *Yeast* (e.g., *Saccharomyces cerevisiae* for fermentation studies).
   - ✓ **Key Feature**: Unicellular organisms that proliferate autonomously in simple nutrient media.

B. **Primary Cultures**
   - ✓ **Definition**: Cells isolated directly from living tissue (e.g., biopsy, organ resection) and cultured *ex vivo*.
   - ✓ **Characteristics**:
     - Genetically and phenotypically identical to the original tissue.
     - Limited lifespan due to the **Hayflick limit** (finite number of divisions before senescence).
     - Used for short-term studies (e.g., drug toxicity testing, personalized medicine).
   - ✓ **Example**: Hepatocytes isolated from a liver biopsy to study metabolic pathways.

C. **Cell Lines**

- ✓ **Definition**: Populations of cells with **immortalized growth** (unlimited division potential).
- ✓ **Types**:
  - **Cancer-derived cell lines**: Naturally immortalized (e.g., HeLa cells from cervical cancer).
  - **Artificially immortalized cells**: Healthy cells modified via:
    - *Viral oncogenes* (e.g., SV40 T-antigen).
    - *Telomerase activation* (extends telomeres to bypass senescence).
  - **Hybridomas**: Fused cells (e.g., antibody-producing B cells + myeloma cells).
- ✓ **Applications**: Long-term experiments, industrial-scale production (e.g., monoclonal antibodies).

D. **Organ Explants**

- ✓ **Definition**: Thin slices or fragments of tissue cultured to preserve 3D architecture and cell-cell interactions.
- ✓ **Optimization**: Thickness adjusted based on tissue oxygenation needs (e.g., 200–400 µm for brain slices).
- ✓ **Use Case**: Studying neuronal networks or tumor microenvironments.

3. Classification of Culture Systems

Cultures are classified based on the **biological material** and **structural complexity**:

3.1. Cell Culture

- **Definition**: Growth of isolated *prokaryotic* (bacteria) or *eukaryotic* (mammalian, plant) cells under **controlled conditions**.
- **Key Features**:

  - ✓ Cells dissociated from tissues (e.g., using trypsin).
  - ✓ Grown as **monolayers** (adherent cells) or **suspensions** (non-adherent cells).
  - ✓ Environment tailored to mimic physiological conditions (pH, temperature, CO_2).

- **Example**: Culturing CHO (Chinese Hamster Ovary) cells for recombinant protein synthesis.

3.2 Tissue Culture

- **Definition**: Maintenance or growth of intact tissues (animal or plant) in vitro.
- **Methods**:
  - **Organotypic culture**: Tissues retain partial architecture (e.g., skin models for burn research).
  - **Histotypic culture**: Cells reorganize into tissue-like structures (e.g., 3D collagen matrices).
- **Applications**:
  - Drug permeability studies (e.g., intestinal epithelial layers).
  - Developmental biology (e.g., embryonic limb bud cultures).

3.3 Organ Culture

- **Definition**: Cultivation of **whole organs** or **large organ fragments** to preserve function and morphology (Table 1).
- **Techniques**:
  - **Air-liquid interface**: For respiratory or skin cultures.
  - **Perfusion systems**: Mimic blood flow (e.g., liver lobule cultures).
- **Challenges**: Maintaining oxygenation and nutrient diffusion in thick tissues.
- **Example**: Culturing embryonic kidneys to study branching morphogenesis.

Table 1 : Critical Distinction between cell culture and organ culture

Aspect	Cell Culture	Organ Culture
Structure	Dissociated cells	Intact 3D architecture
Lifespan	Immortalized or primary	Short-term (days to weeks)
Complexity	Low (single cell type)	High (multiple cell interactions)
Applications	Molecular mechanisms, biomanufacturing	Developmental studies, disease modeling

❖ **Notes for Learners**

- ✓ **Primary vs. Cell Lines**: Primary cultures reflect *in vivo* physiology but have limited lifespan; cell lines offer reproducibility but may accumulate genetic drift.
- ✓ **Organ Culture Limitations**: Requires specialized equipment (e.g., bioreactors) and is labor-intensive.
- ✓ **Ethical Considerations**: Use of human-derived cells necessitates informed consent and ethical oversight.

4. Types of Cell Cultures

4.1 Primary Culture (Primoculture)

4.1.1 Definition

A primary culture is established by isolating cells, tissues, or organs directly from a living organism (Figure 1). These cultures retain the biological properties of the original tissue but have a finite lifespan due to replicative senescence.

4.1.2 Key Characteristics

1. **Initiation**:
    - Requires enzymatic (e.g., collagenase, trypsin) or mechanical dissociation of tissues.
    - Example: Isolating hepatocytes from a liver biopsy.
2. **Growth Limitations**:
    - **Nutrient Depletion**: Proliferation ceases when critical nutrients (e.g., glucose, growth factors) are exhausted.
    - **Contact Inhibition**: Adherent cells stop dividing once they form a **confluent monolayer** (a continuous cell sheet covering the substrate).
3. **Lifespan**:
    - Typically survives for 5–10 population doublings (e.g., human fibroblasts).

4.1.3 Challenges

1. **Heterogeneity**: Mixed cell populations (e.g., fibroblasts, epithelial cells).

2. **Contamination Risks**: Higher susceptibility to microbial infection without antibiotics.
3. **Ethical Constraints**: Requires fresh tissue from biopsies or surgeries.

4.1.4 Applications

- Short-term studies: Drug toxicity screening, viral replication assays.
- Personalized medicine: Testing patient-specific tumor cell responses.

❖ **Note**

Primary cultures must be **subcultured** (passaged) before reaching confluence, but their limited replicative capacity restricts long-term use.

Tissue isolation → 2. Dissociation → 3. Plating → 4. Confluent monolayer.

Figure 1 : Primary Culture Workflow

4.2 Secondary Culture (Subculture)

4.2.1 Definition

When primary culture cells reach confluence, they are harvested, diluted, and transferred to fresh medium in a new vessel. This process, called **subculturing** or **passaging**, generates secondary cultures (Figure 2).

4.2.2 Key Steps

- ✓ **Detachment**: Enzymatic treatment (e.g., trypsin-EDTA) dissociates adherent cells.
- ✓ **Reseeding**: Cells are diluted (e.g., 1:4 ratio) and replated with fresh medium.

4.2.3 Characteristics

- ✓ **Genetic Stability**: Retains most genetic traits of the original tissue.
- ✓ **Senescence**: Secondary cultures eventually undergo replicative senescence (Hayflick limit).

4.2.4 Example

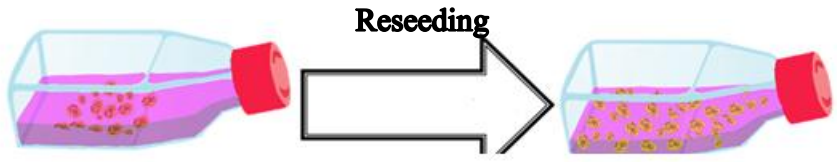
Human keratinocytes from skin biopsies can be subcultured 2–3 times before senescence.

4.2.5 Advantages Over Primary Cultures

- ✓ Extended usability for repeated experiments.
- ✓ Reduced contamination risk with established aseptic techniques.

4.2.6 Limitations

- ✓ Gradual loss of specialized functions (e.g., hepatocyte albumin production).
- ✓ Accumulation of genetic abnormalities over passages.



Confluent primary culture → 2. Trypsinization → 3. Reseeding → 4. Secondary culture proliferation.

Figure 2 : Subculturing Process

5.3 Cell Lines

5.3.1 Definition

A cell line is a homogeneous population of **immortalized cells** capable of indefinite proliferation *in vitro* through genetic or epigenetic modifications (Table 2).

5.3.2 Origins

A. **Spontaneous Tumors**:
   - ✓ Derived from cancerous tissues (e.g., HeLa cells from Henrietta Lacks' cervical carcinoma).

B. **Artificial Immortalization**:
   - ✓ **Viral Oncogenes**: SV40 T-antigen or HPV E6/E7.
   - ✓ **Telomerase Activation**: Overexpression of hTERT (human telomerase reverse transcriptase).

5.3.3 Key Features

- ✓ **Unlimited Proliferation**: Bypasses senescence via genetic alterations.
- ✓ **Standardization**: Genetically uniform populations for reproducible experiments.

5.3.4 Applications

- ✓ Biomanufacturing: Monoclonal antibodies (e.g., hybridomas), vaccines.
- ✓ Cancer research: Drug resistance studies using HeLa cells.

5.3.5 Examples

- ✓ **HeLa**: First immortal human cell line (polio vaccine development).
- ✓ **HEK293**: Human embryonic kidney cells modified with adenovirus DNA.

5.3.6 Ethical Considerations

- ✓ Tissue ownership controversies (e.g., Henrietta Lacks' case).
- ✓ Regulatory oversight for commercial use.

5.3.7 Challenges

- ✓ **Genetic Drift**: Mutations alter behavior over time.
- ✓ **Cross-Contamination**: Misidentification (e.g., HeLa contamination).

Table 2: Comparison of Culture Types

Aspect	Primary Culture	Secondary Culture	Cell Line
Lifespan	Days to weeks	Weeks to months	Indefinite
Genetic Stability	High	Moderate	Low (drift over time)
Applications	Short-term studies	Extended experiments	Industrial-scale production

Chapter 2: Infrastructure, Equipment, and Sterilization Techniques

1. Introduction to laboratory equipment

The specific requirements of a cell culture laboratory depend largely on the type of research conducted. However, all cell culture work demands **absolute sterility**, as microbial contamination can lead to cell lysis and experimental failure. For this reason, every cell culture laboratory must adhere to strict **aseptic conditions** to exclude pathogenic microorganisms. While research goals vary, these facilities share essential equipment and protocols to ensure cell viability, reproducibility, and contamination-free environments.

A functional cell culture laboratory relies on several critical tools and systems:

- **Microbiological safety cabinets (MSCs) or laminar flow hoods**: These provide a sterile workspace by filtering air through HEPA filters, creating a contamination-free zone for handling cells and reagents.
- **Incubators**: Maintain optimal growth conditions (e.g., 37°C, 5% CO_2, and controlled humidity) to mimic the cells' natural environment.
- **Culture media**: Formulated to meet the nutritional needs of specific cell types, often supplemented with growth factors, vitamins, and serum.
- **Refrigerators (+4°C)**: Store temperature-sensitive reagents, media, and short-term cell samples.
- **Freezing media**: Cryoprotective solutions (e.g., containing DMSO) to preserve cell viability during freezing.
- **Ultra-low temperature freezers (-80°C) or liquid nitrogen tanks**: Long-term storage for cryopreserved cell lines.
- **Refrigerated centrifuges**: Maintain low temperatures during cell pelleting or reagent preparation to prevent degradation.
- **Inverted microscopes**: Enable real-time observation of adherent cell monolayers from beneath the culture vessel, critical for monitoring morphology and confluency.

1.1 Microbiological Safety Cabinet (MSC)

A microbiological safety cabinet (MSC) is an enclosed workspace designed to protect both the user and the environment from hazards associated with aerosols. It creates a barrier that prevents external particles from entering the workspace while maintaining a constant flow of

filtered air. This airflow safeguards the working area from dust and microbial contaminants. Ambient air is filtered through a **HEPA (High-Efficiency Particulate Air) filter**, which removes 99.97% of particles ≥0.3 µm in size.

There are two main types of laminar flow hoods:

1.1.1 Type I Hood

In a Type I hood, the workspace is fully protected by the surrounding airflow. This type of cabinet directs a vertical laminar airflow through a HEPA filter. However, the operator is not fully protected, as **20% of the vertical airflow exits the cabinet at the front**, while the remaining **80% is recirculated** (Figure 3).

The Type I hood provides **limited operator protection**, as a portion of the airflow escapes into the room, potentially exposing the user to contaminants. However, it offers **high workspace protection** due to its continuous **HEPA-filtered airflow**, which creates a sterile environment for cell culture work. This makes the Type I hood particularly suitable for handling **low to moderate-risk materials**, where the primary focus is on protecting the sample rather than the operator. It is commonly used in applications where the risk of hazardous aerosols is minimal, such as routine cell culture maintenance or non-infectious material handling.

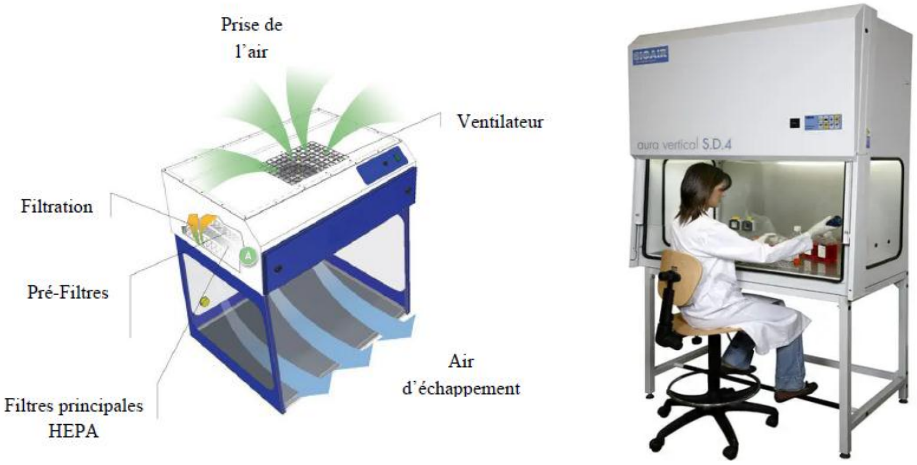


Figure 3 : Vertical Laminar Flow HEPA Hood.

1.1.2 Type II Hood

In a Type II hood, the **operator**, **product**, and **environment** are all fully protected. The airflow system is designed to ensure safety and sterility:

- **Airflow Mechanism**:
  - ✓ **70% of the air** is recirculated through a HEPA filter located at the top of the hood.
  - ✓ **30% of the air** is exhausted to the outside after passing through a second HEPA filter, ensuring environmental protection.
- **Operator Protection**: A **front intake airflow** creates a barrier that prevents contaminants from escaping into the workspace, while also protecting the user from exposure.

The Type II hood, or Microbiological Safety Cabinet (MSC), offers **triple protection**, safeguarding the **operator**, the **sample**, and the **environment**. This is achieved through a sophisticated airflow system that combines **recirculation** and **exhaust mechanisms**. Dual **HEPA filters** play a critical role, ensuring sterile conditions by removing **99.97% of particles ≥0.3 µm** in size. These features make the Type II hood indispensable for handling **high-risk materials**, such as **primate and human cell lines**, as well as **viral vector production** or **infection studies**. Its design ensures both operational safety and experimental integrity, making it a cornerstone of modern cell culture laboratories.

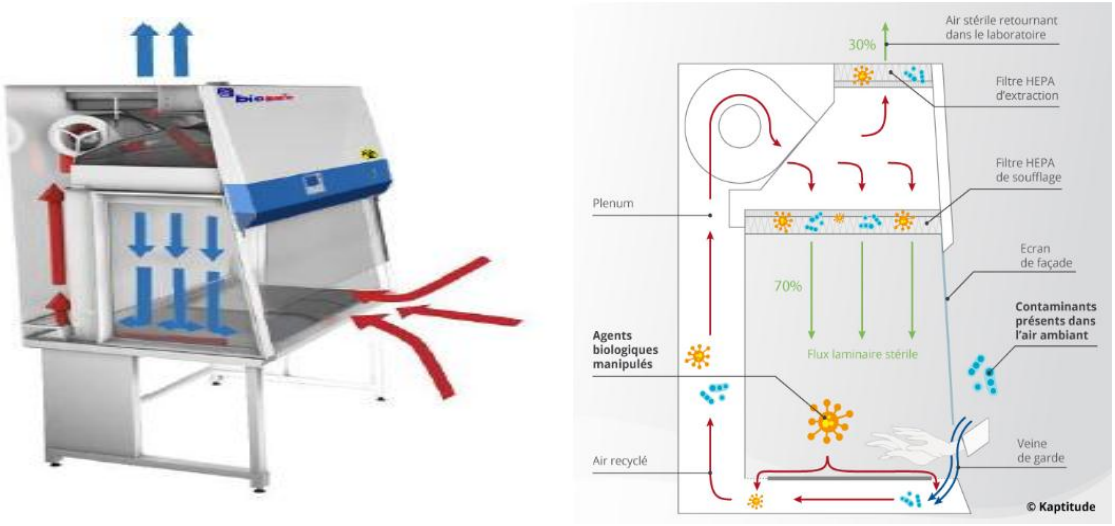


Figure 4 : Type II Hood or Microbiological Safety Cabinet (MSC).

1.2 Incubators and Their Role

Incubators are essential equipment in cell culture laboratories, designed to maintain optimal conditions for cell growth and viability. The most commonly used type is the **CO_2 incubator**, which features a **water jacket** and **heated door**.

- **Heated Door**: Prevents condensation on the inner glass door, ensuring clear visibility of the cultures without the need to open the incubator, which could disrupt the internal environment.
- **Water Jacket**: Provides an airtight and humidified chamber, minimizing water evaporation from culture media. This helps maintain consistent humidity levels, which is critical for cell health.

These incubators operate at **37°C** (mimicking human body temperature) and **5% CO_2** to maintain the pH of the culture medium at approximately **7.4**, which is ideal for most mammalian cells. The CO_2 reacts with the bicarbonate buffer in the medium to stabilize the pH, ensuring proper cellular function.

Modern CO_2 incubators are equipped with **digital displays** and **sensors** to monitor and record temperature and gas levels. They also feature **alarm systems** that alert users if conditions deviate from the set parameters, such as a drop in temperature or CO_2 concentration. This ensures timely intervention to prevent damage to sensitive cultures.

CO_2 incubators are indispensable for a wide range of applications in cell culture laboratories. They are primarily used for **culturing mammalian cells**, which are essential for **research**, **drug development**, and **biomanufacturing** processes. These incubators provide the stable environment needed to grow and maintain cells for experiments, toxicity testing, and the production of biologics. Additionally, they are critical for **maintaining stem cells**, **primary cultures**, and **cell lines** under tightly controlled conditions.

Figure 5 : CO_2 Incubator with Water Jacket and Heated Door.

1.3 Centrifuges

Centrifuges are essential equipment in every cell culture area, and **refrigerated centrifuges** are particularly important for handling temperature-sensitive biological samples. When working with human cells, it is crucial to use **sealed rotors** to prevent aerosol formation, which could pose a contamination risk or health hazard. A centrifugal force of **100 x g** is typically sufficient to sediment cells without causing damage. However, higher g-forces can lead to cell rupture or loss of viability, so care must be taken to select the appropriate speed for each cell type. Centrifuges are commonly used for tasks such as pelleting cells after trypsinization, separating cellular components, or concentrating cell suspensions for downstream applications.

1.4 Refrigerators and Freezers

Refrigerators and freezers are critical for the proper storage of cell culture reagents and samples.

- **Refrigerators (+4°C)**: These are used to store **liquid media**, **buffers**, and temperature-sensitive reagents such as **enzymes** (e.g., trypsin) and **medium**

components (e.g., glutamine and serum). A dedicated refrigerator or cold room ensures that these materials remain stable and effective for use.

- **Freezers (-20°C)**: These are ideal for storing pre-aliquoted stocks of **serum**, **nutrients**, and **antibiotics**, which are sensitive to repeated freeze-thaw cycles. Freezers at this temperature are also suitable for long-term storage of certain reagents.
- **Ultra-Low Temperature Freezers (-70°C or lower)**: For long-term preservation of **cell stocks**, ultra-low temperature freezers or **liquid nitrogen tanks** are required. These systems prevent ice crystal formation, which can damage cell membranes and compromise viability. Liquid nitrogen storage, in particular, is the gold standard for maintaining cell lines indefinitely, as it keeps samples at temperatures below -150°C.

1.5 Microscopes

A **simple inverted microscope** is an indispensable tool in cell culture laboratories, allowing researchers to examine cultures directly in flasks, plates, or other containers without disturbing them. This type of microscope is particularly useful for observing **cell morphology**, **confluency**, and **growth patterns**. Recognizing changes in cell morphology is critical, as these can serve as the first indication of **culture deterioration**, such as contamination, stress, or senescence. For example, healthy adherent cells typically exhibit a uniform, spread-out appearance, while stressed or dying cells may appear rounded or detached (Figure 6).

For routine tasks like **cell counting** using a hemocytometer, a basic optical microscope with **100x magnification** is sufficient. However, more advanced techniques, such as **chromosomal analysis** or **autoradiography**, require higher-quality microscopes with enhanced resolution and imaging capabilities. These advanced microscopes may include features like phase contrast, fluorescence, or differential interference contrast (DIC) to provide detailed insights into cellular structures and functions.

Figure 6 : Inverted Microscope.

1.6 Liquid Nitrogen Freezer

For both **continuous** and **finite cell lines**, freezing and storing cell samples is a critical practice to ensure long-term preservation. Maintaining a consistent supply of frozen cell stocks helps prevent **genetic drift**—a gradual change in the genetic makeup of cells over time—and safeguards against the loss of valuable cell lines due to contamination or other issues.

The freezing process is generally standardized for all cultured cells. Cells should be harvested during their **exponential growth phase**, when they are most viable, and mixed with a **cryoprotectant** such as **dimethyl sulfoxide (DMSO)**. DMSO prevents ice crystal formation, which can damage cell membranes during freezing. The cells are then frozen slowly at a controlled rate of **1°C per minute** until they reach **-50°C**, after which they are transferred to **liquid nitrogen** for long-term storage at **-196°C**. Cells can be stored either **immersed in liquid nitrogen** (in sealed glass ampoules) or in the **vapor phase** above the liquid nitrogen (in screw-cap vials). Both methods ensure the preservation of cell viability and functionality for years.

1.7 Tissue Culture Containers

A wide variety of tissue culture vessels are available, with the most common being **polystyrene containers** treated for cell adhesion. These include:

- **Petri dishes**: Suitable for small-scale cultures or experiments requiring easy access to cells.
- **Flasks** (e.g., 25 cm^2 or 75 cm^2): These are widely used for routine cell culture and offer the added advantage of being **gas-tight**. After gassing with CO_2, the flasks can be sealed, eliminating the need for a CO_2 incubator. This feature is particularly useful in emergencies, such as incubator failure (Figure 7).

Multi-well plates (e.g., 6-well, 12-well, or 96-well formats) are also commonly used for high-throughput experiments, drug screening, or assays requiring multiple replicates. The choice of container depends on the scale of the experiment and the specific requirements of the cell type being cultured.

Figure 7 : Left: Multi-well plate (6 wells). Right: Culture flask.

2. Sterilization Methods :

All procedures required for cell culture necessitate working in a sterile environment, meaning an environment free from any living microorganisms. To achieve this, various treatments exist to sterilize the equipment needed for the work.

2.1 Physical Methods

2.1.1 Heat Sterilization

A. Dry Heat:

Dry heat works by denaturing enzymes and nucleic acids through oxidation, which kills the microorganism. This method is particularly effective for heat-resistant materials and is widely used in laboratories and medical settings.

❖ **Flaming (Direct Heating):**

This method is based on the use of a Bunsen burner. It is used for the immediate sterilization of handling equipment, ensuring that tools are free from contaminants before use. It is suitable for glassware (pipettes, test tubes, petri dishes, etc.) and certain metal objects (inoculation loops, forceps, scalpels, etc.).

It should be noted that the Bunsen burner, adjusted to produce a blue flame (the hottest part of the flame), creates a sterile zone with a diameter of approximately 20 cm. This zone is essential for maintaining aseptic conditions during microbiological work. All manipulations involving the opening of culture tubes and plates, as well as inoculation, should be performed within this sterile zone.

To ensure this zone remains sterile, air currents and movement of people must be avoided, as they can introduce contaminants. The Bunsen burner should be placed on a stable, heat-resistant surface, and the flame should be adjusted to a consistent blue flame for optimal heat output. Additionally, tools should be passed through the flame repeatedly to ensure thorough sterilization, and care should be taken to avoid overheating or damaging delicate instruments **(Figure 8).**

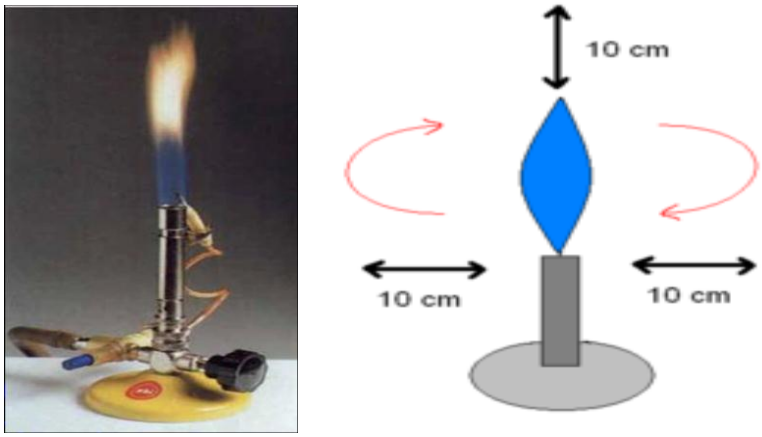


Figure 8 : Bunsen burner.

❖ **The Pasteur Oven, Poupinel (Hot Air Sterilizers):**

This is a hot air oven that utilizes dry heat for sterilization. It is commonly used for sterilizing empty glassware (such as test tubes, Petri dishes, culture tubes and stoppers, Pasteur pipettes, and various containers), as well as porcelain and metal instruments (Figure 9).

The glassware to be sterilized must be thoroughly cleaned and completely dry. It can optionally be plugged with cotton and wrapped in sturdy, heat-resistant paper to maintain sterility after the process. Dry heat sterilization works by destroying spores and microorganisms through oxidation. The recommended heating parameters are:

- ✓ 30 minutes or more at 180°C.
- ✓ 1 hour or more at 170°C.
- ✓ 2 hours or more at 160°C.

After sterilization, the equipment should remain in the oven until it has cooled completely to avoid contamination from sudden exposure to non-sterile air. Once cooled, the sterilized materials should be stored in a clean, dust-free environment to maintain their sterility until use.

Figure 9 : The Poupinel Oven (Pasteur Oven).

B. Moist Heat:

❖ **The Autoclave:**

This technique involves boiling water in a sealed chamber to increase pressure, allowing heating to occur under steam pressure at temperatures ranging from 100°C to 130°C. The

duration of sterilization varies depending on the medium, the temperature used, and the volume of the containers (minimum 15 minutes) (Figure 10).

The autoclave is used to sterilize both new and contaminated culture media, as well as other microbiological materials such as rubber equipment, glassware, and more. In a moisture-saturated environment under pressure, sterilization occurs at lower temperatures compared to dry heat methods.

Before each sterilization cycle, the water level in the autoclave must be checked. The materials to be sterilized are placed in the autoclave's metal baskets. Containers should be plugged with cotton (avoid handling the cotton with fingers—use forceps instead) and covered with aluminum foil or sturdy wrapping paper to maintain sterility.

After sterilization, the materials should be allowed to cool inside the autoclave before removal to prevent contamination. Properly sterilized items are then stored in a clean, dust-free environment until use.

Figure 10 : Autoclave.

- **The Water Bath or Thermostatic Bath (Boiling):**

Heating for 30 minutes at 100°C by boiling or maintaining in boiling steam is sufficient to destroy all vegetative forms of microorganisms (Figure 11).

This method is particularly suitable for sterilizing delicate liquid products, such as albuminous media, milk, gelatin, concentrated carbohydrate solutions, and other heat-sensitive substances. The water bath provides a controlled and gentle heating environment, ensuring that the integrity of the materials is preserved while achieving effective sterilization.

Figure 11 : Water bath Bain marie.

C. Other Methods:

Some fragile culture media cannot withstand high temperatures, so boiling at 100°C is used as an alternative.

- **Pasteurization:**

Pasteurization is always followed by rapid cooling. It can be carried out in bottles. This method is used for the short-term preservation of natural products, as it destroys only vegetative forms of microorganisms but not spores. The liquid is quickly heated to 90°C for 30 seconds, for example, and then abruptly cooled to 10°C.

Pasteurization is widely used in the food industry for preserving products such as milk, beer, fruit juices, and other perishable liquids. While it does not achieve complete sterilization, it significantly reduces microbial load, extending the shelf life of the products.

- **Tyndallization:**

Tyndallization involves a series of brief heatings at temperatures of 70°C at regular intervals (e.g., three heatings of one hour each, with 24 hours between each heating). This process allows resistant forms, such as spores, to germinate so they can be killed in the subsequent heating cycle.

This method is used to eliminate both vegetative forms (bacteria) and resistant forms (spores). The discontinuous heating at low temperatures is sufficient to kill vegetative forms while

inducing thermal shock, which triggers the germination of resistant forms. These newly germinated forms are then destroyed in the next heating cycle.

Tyndallization is particularly useful for sterilizing fragile media containing serum (e.g., vaccines), egg yolk, or other thermosensitive, highly viscous substances that cannot be sterilized by filtration. It provides a gentle yet effective way to achieve sterility without damaging heat-sensitive components.

2.1.2 Filtration Sterilization

Filtration sterilization involves passing the liquid to be sterilized through a porous wall or membrane that retains bacteria and other microorganisms. This method is particularly useful for heat-sensitive media, but it is only feasible when the viscosity of the media is low.

Filtration is widely used in laboratories and industries to sterilize liquids that would be damaged by heat, such as solutions containing heat-labile components. Several types of filtration systems are available (Figure 12):

- **Porcelain Candles (Chamberland Type):** These are rounded-bottom tubes made of porous porcelain. The pore size of these filters ranges from a few micrometers to one-tenth of a micrometer. They are durable and reusable after proper cleaning and sterilization.
- **Filter Discs:** These are made of sintered glass with pore sizes ranging from 150 to 1 micrometer. They are commonly used for filtering small volumes of liquids and are suitable for both sterilization and clarification purposes.
- **Disposable Membrane Filters:** These are thin plastic membranes containing millions of pores per square centimeter. The pore size is highly uniform and can range from 8 to 0.01 micrometers. These membranes are the most commonly used filters in modern laboratories due to their convenience, reliability, and ability to achieve high levels of sterility.
- **Sterilization of Heat-Sensitive Liquids:** Filtration is ideal for sterilizing liquids that are heat-labile, such as carbohydrate solutions, vitamins, enzymes, and other biologically active compounds. This method ensures that the integrity and functionality of these sensitive substances are preserved.

Filtration sterilization is a critical technique in microbiology, pharmaceuticals, and biotechnology, as it allows for the removal of microorganisms without exposing the media to high temperatures. Proper selection of the filter type and pore size is essential to achieve effective sterilization while maintaining the quality of the filtered liquid.

Figure 12 : Different types of filters.

2.1.3 Radiation Sterilization

A. Sterilization by UV Radiation:

UV radiation is the most commonly used method in laboratories for air and surface decontamination, particularly for workbenches located under protective hoods. UV radiation acts directly on microorganisms by damaging their DNA, but its penetration is limited, making it suitable only for surface sterilization.

This method is widely used in virology, cell culture, pharmaceutical preparation and packaging, bacterial inoculation, and media preparation. Instruments or containers such as Petri dishes can also be sterilized using UV radiation, provided they are exposed directly to the light source. However, it is important to note that UV radiation does not penetrate materials like plastic or glass, so it is only effective for surfaces directly exposed to the light.

- **Applications of UV Sterilization:**

- ✓ Decontamination of laboratory air and surfaces.
- ✓ Sterilization of Petri dishes and other small equipment.

- ✓ Prevention of contamination in cell culture and virology work.
- ✓ Preparation of sterile media and solutions.

B. Other Radiations (X-rays, etc.):

Other forms of radiation, such as X-rays and gamma rays, are less commonly used but can be highly effective for industrial sterilization processes. For example, ionization radiation is used for sterilizing plastic Petri dishes and other heat-sensitive materials. This method is also employed in the food industry for the preservation of certain products, as it effectively kills microorganisms without significantly altering the product's quality.

❖ **Applications of Ionization Radiation:**

- ✓ Industrial sterilization of plastic Petri dishes and medical devices.
- ✓ Preservation of food products by eliminating pathogens and extending shelf life.
- ✓ Sterilization of heat-sensitive materials that cannot withstand traditional heat-based methods.

Radiation sterilization, whether by UV or ionization, offers a non-thermal alternative for achieving sterility, making it invaluable in fields where heat-sensitive materials or products are involved.

2.2 Chemical Methods

Chemical methods are generally used for disinfecting rooms, work surfaces, and for destroying germs carried by contaminated instruments. This mode of sterilization is systematically practiced in laboratories, particularly for slides and glassware that cannot be autoclaved. Chemical agents are essential for maintaining a sterile environment and preventing contamination in sensitive areas.

2.2.1 Antiseptics

Antiseptics are substances that kill living cells, including microorganisms. Their action is instantaneous, making them ideal for immediate disinfection. Examples include iodine tincture, potassium permanganate, hydrogen peroxide, and others. They are primarily used locally on living organisms, such as on wounds and mucous membranes.

- ✓ **Ethyl Alcohol (60%):** Used for disinfecting workbenches and instruments. However, some germs are resistant to alcohol, so disinfection is not always guaranteed.
- ✓ **Sodium Hypochlorite (Bleach):** Diluted to 1/4 strength, it is used in containers for used slides or in spray bottles for disinfecting hands, workbenches, and floors.
- ✓ **Soaps and Detergents:** These act primarily through their wetting power, which helps remove germs mechanically from surfaces.

2.2.2 Disinfectants

Disinfectants prevent contamination in human and animal environments. They can be in the form of vapors (e.g., formaldehyde, sulfur dioxide, ethylene oxide) or liquids (e.g., phenols, copper sulfate, iron sulfate, bleach). Disinfectants are used for all external environments, such as water, air, and soil.

- ✓ **Formaldehyde Vapor:** Produced by heating a formaldehyde solution, it is used to disinfect rooms and incubators.
- ✓ **Ethylene Oxide:** Used in the industry for sterilizing certain single-use plastic materials. It is highly effective but requires careful handling due to its toxicity.

2.2.3 Antibiotics

Antibiotics are divided into two types:

- ❖ **Bacteriostatic Antibiotics:** These stop bacterial multiplication without killing the bacteria.
- ❖ **Bactericidal Antibiotics:** These kill bacteria directly.

Antibiotics are generally used for in vivo cultures and treatments. They play a critical role in controlling bacterial infections in both medical and laboratory settings.

Table 3: Disadvantages of Sterilization Methods

Treatment and Description	Possible Disadvantages
Flame Treatment: The equipment is heated over a flame. Microorganisms are killed by the heat.	The equipment may melt under the heat of the flame.
Dry Heat Oven Treatment: The equipment is heated inside an oven, which kills microorganisms.	Equipment that cannot withstand heat may break.
Chemical Treatment: The equipment is soaked in a solution or exposed to a gas, which kills microorganisms.	The substances used are often harmful to human health and difficult to handle safely.
Steam Treatment: This treatment is usually done in an autoclave, a device with a sealed chamber where the equipment to be sterilized is placed. High pressure and heat kill the microorganisms.	Equipment that cannot withstand moisture cannot be sterilized with steam.
Radiation Treatment: The equipment is exposed to radiation (X-rays, UV rays, gamma rays, etc.) that kills microorganisms.	Exposure to radiation can be harmful to human health.

2.3 Sterility in Cell Culture

The concept of sterility in cell culture can sometimes be challenging to grasp. In essence, something that is "sterile" contains no living organisms. However, in practice, a "sterile" cell culture refers to a culture that contains only the desired cells and no other living organisms.

2.3.1 Sterilization of Culture Media

Everything that must be free of any cells must be sterilized. This is typically achieved through filtration under sterile conditions (e.g., under a flame or in a laminar flow hood) using a membrane filter with a pore size of 0.22 μm (e.g., a Millipore filter). Filters come in various types and sizes, and most require pressure to function (e.g., vacuum pumps or water aspirators). A 0.22 μm filter retains all bacteria and fungi, allowing only viruses to pass through.

Autoclaving (exposure to high temperatures, e.g., 121°C for 20 minutes) is possible for some solutions, but this method cannot be used for culture media containing proteins, as the high heat would denature or "cook" them.

2.3.2 Sterilization of the Culture

The biologist's greatest fear is the contamination of cell cultures. When a culture is colonized by another organism, the desired cells are severely disrupted. The contaminant induces stress in the cells through competition for nutrients, as it often proliferates faster, and through danger signals, which profoundly alter the cells' behavior.

The most common contaminants are bacteria from our skin, such as *E. coli* and *S. aureus*, as well as certain yeasts. Contaminations by *Mycoplasma* species are also frequent. Additionally, cross-contamination with other cell types can occur, especially when the same person works on multiple cell lines simultaneously in the same location.

Maintaining sterility in cell culture requires strict adherence to aseptic techniques, regular monitoring for contaminants, and proper sterilization of all equipment and media. This ensures the integrity and reproducibility of experimental results.

2.3.3 Sterilization of Surfaces

The clean zone must be kept as clean as possible through regular decontamination using a surface decontaminant cleaner. As a precaution, all surfaces entering the clean zone should also be cleaned and decontaminated, including bottles, flasks, gloves, hands, and any other equipment or materials. This ensures that no contaminants are introduced into the sterile environment, which is critical for maintaining the integrity of cell cultures.

2.3.4 Sterilization of Equipment

The availability of a wide range of plastic tissue cultureware has significantly reduced the need for traditional glassware. However, glass items such as pipettes still require thorough cleaning and sterilization.

- ✓ **Cleaning Process:** Glassware must first be soaked in an appropriate detergent, followed by a rigorous washing procedure. This includes a complete rinse in distilled water to remove any detergent residues before drying and sterilization.
- ✓ **Preparation for Sterilization:** Pipettes are often plugged with non-absorbent cotton wool before sterilization to prevent contamination during use.

- ✓ **Sterilization of Glassware:** Items such as pipettes, conical flasks, and beakers (covered with aluminum foil) are sterilized in a hot air oven at 160°C for one hour.
- ✓ **Sterilization of Other Equipment:** Additional equipment, such as automatic pipette tips and bottles (with loosened caps), is autoclaved at 121°C for 20 minutes.

Proper sterilization of equipment is essential to prevent contamination and ensure the reliability of cell culture experiments. Regular maintenance and adherence to sterilization protocols help maintain a sterile working environment.

Chapter 3:
Culture Media: Composition and Preparation

1. Composition of Culture Media:

Culture media must replicate, as closely as possible, the conditions of the environment that cells experience in vivo. Therefore, they must serve the following purposes:

- ✓ Act as a carrier of nutrients.
- ✓ Maintain physicochemical conditions such as pH and osmolarity.
- ✓ Support cell proliferation (cell division).

For long-term cultures, changing the culture media every 2 to 3 days is essential. Culture media are available from many suppliers in either powdered or liquid form.

A culture medium can consist of:

- ❖ A combination of a synthetic basal medium + serum (the most effective being fetal bovine serum).
- ❖ Or, a defined medium = synthetic medium without serum.

A culture medium must contain the minimal cellular requirements:

- ✓ **Water**
- ✓ **Mineral ions** (for osmolarity)
- ✓ **Carbon and energy sources**: Glucose
- ✓ **Amino acid sources**
- ✓ **Buffer solution** (to regulate pH to 7.4)
- ✓ **Phenol red**: pH indicator
- ✓ **Vitamins**: Enzyme cofactors
- ✓ **Nitrogenous bases**: Ribose and deoxyribose

Additionally, a culture medium contains variable supplements depending on the medium:

- ✓ **Glutamine** at 1%
- ✓ **Fetal bovine serum (FBS)**
- ✓ **EGF (Epidermal Growth Factor)**
- ✓ **Adhesion factors**
- ✓ **Antitrypsin inhibitors**

- ✓ **Antibiotic mixture** at 1% (Penicillin, Streptomycin, and Antifungal agents)

1.1 Synthetic Basal Media:

Basal media are entirely synthetic, with compositions ranging from simple to complex. They are also referred to as MEM = Minimum Essential Medium. These media are considered "basal" because they only ensure the survival of cells in vitro.

To support cell proliferation and the expression of various cellular functions in culture, a certain concentration of serum must be added to the synthetic basal medium.

Synthetic basal media must contain:

- ✓ **Essential nutrients** for cell survival.
- ✓ **Balanced salts** to maintain osmotic pressure.
- ✓ **Buffering agents** to stabilize pH.
- ✓ **Energy sources** such as glucose.

These media serve as the foundation for cell culture, and their composition can be customized with additional supplements to meet the specific needs of different cell types.

1.1.1 Mineral Salts:

Seven essential ions are required in culture media: Na^+ (sodium), K^+ (potassium), PO_4^{3-} (phosphate), Mg^{2+} (magnesium), Ca^{2+} (calcium), Cl^- (chloride), and CO_3^{2-} (carbonate). Some media also include trace metals such as iron, copper, cobalt, and selenium.

- ❖ **Role:**

- ✓ Serve as cofactors for enzymes.
- ✓ Contribute to osmotic pressure regulation.
- ✓ Help regulate membrane potential by providing sodium, potassium, and calcium ions.
- ✓ Facilitate cellular communication and pH regulation.
- ✓ Aid in cell attachment (particularly Ca^{2+}).
- ✓ Are integral components of certain molecules.

1.1.2 Amino Acids:

There are eight essential amino acids for humans: Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Threonine (Thr), Tryptophan (Trp), and Valine (Val).

Note: Glutamine (Glu) is particularly important for almost all mammalian cell cultures. It serves as a precursor for the synthesis of purines, pyrimidines, and some amino acids. A deficiency in glutamine can lead to halted cell growth due to inhibited DNA synthesis.

- ❖ **Role:**

- ✓ Amino acids are the building blocks of proteins.
- ✓ Essential amino acids must be included in the culture medium because cells cannot synthesize them on their own.

1.1.3 Vitamins:

Vitamin requirements vary depending on the cell type. According to Eagle, eight vitamins are essential: Choline (classified as a B vitamin), Folic Acid (B9), Thiamine (B1), Pyridoxal (B6), Riboflavin (B2), Nicotinic Acid (B3), Inositol (B7), and Pantothenic Acid (B5).

- ❖ **Role:**

- ✓ Act as enzyme cofactors or precursors for the synthesis of molecules.

1.1.4 Glucose:

The primary energy source in culture media is typically glucose, at a concentration of 1 g/L, similar to that found in human serum. Glucose is sometimes substituted with other sugars such as galactose, mannose, or fructose.

- ❖ **Role:**

- ✓ Serves as an energy source (catabolism) for cell proliferation.
- ✓ Provides carbon for anabolic processes.

1.1.5 Buffer System

There are two main options for maintaining pH in culture media:

- **Sodium Bicarbonate and Carbon Dioxide System:**

✓ Sodium bicarbonate ($NaHCO_3$) is added to the medium.
✓ Carbon dioxide (CO_2) at a concentration of 5% is maintained in the incubator atmosphere.
✓ This system mimics the physiological conditions of mammalian cells, which are adapted to a stable pH environment.

- Organic Buffer (HEPES):

✓ HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) is an organic buffer added directly to the medium.
✓ It is particularly useful in situations where CO_2 control is difficult, such as in open systems or during transport.

- **Role:**

✓ The buffer system regulates the pH, which is crucial for mammalian cells that originate from an organism with strict homeostatic conditions.
✓ To monitor pH changes, a pH indicator such as phenol red is added to the medium.

1.1.6 Phenol Red:

Phenol red is a widely used pH indicator in cell culture media. It provides a visual indication of pH changes during the culture process.

- **Role:**

✓ Allows for easy visualization of pH variations.
✓ Its color change range is well-suited for the pH levels required in cell culture (around 7.4).

  - At low pH (acidic conditions), the medium turns yellow.

- At high pH (alkaline conditions), the medium turns purple.
- At the optimal pH of 7.4, the medium appears orange-red (Figure 13).

Phenol red is a valuable tool for ensuring that the culture environment remains within the appropriate pH range, which is critical for cell viability and function.

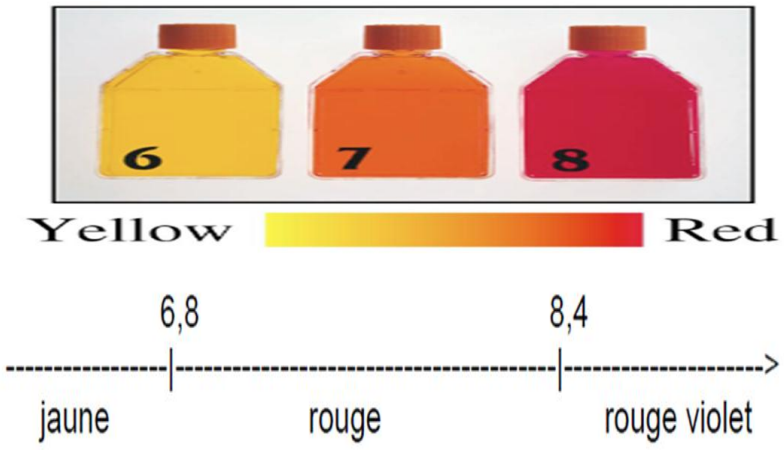


Figure 13 : pH indicator (phenol red).

❖ **Cause of Phenol Red Color Change:**

✓ A properly regulated medium should display an orange-red color.

✓ A purple-red color indicates a pH that is too basic. This is a sign of cell mortality, as lysed cells release proteins that alkalize the medium.

✓ A yellow color indicates significant acidification. This acidification occurs when the cell population becomes too large, resulting from the dissolution of carbon dioxide produced during cell respiration. A yellowing medium necessitates immediate cell subculturing. If the acidification of the medium is accompanied by cloudiness, it is a sign of bacterial contamination.

1.2 Serum:

When cultured in the presence of a synthetic basal medium as described above, most cells are generally only capable of survival. The initiation of cell division requires the presence of certain mitogenic factors, most often provided by serum. The percentage of serum added to the basal medium varies from 2% to 20%, depending on the cell type.

Serum can be derived from humans or young animals, as the overall cytostimulatory effect of serum is inversely proportional to the age of the donor. The most commonly used sera include calf serum, newborn calf serum, and fetal bovine serum (FBS), with the latter offering the best performance. Currently, there are mixtures available that can replace FBS, such as amniotic fluid enriched with specific components.

- **Note:** The addition of serum to culture media must be done extemporaneously (immediately before starting the culture) and under strictly sterile conditions. This ensures the integrity and effectiveness of the serum in supporting cell growth and proliferation.

1.2.1 Fetal Bovine Serum (FBS): Constituents and Roles

A. Growth Factors and Hormones:

- **Growth Factors:** These are peptides involved in the regulation of the cell cycle (chemical messengers). They have a mitogenic effect, promoting cell proliferation.
- **Hormones:** These are essential messengers required for the establishment of specific cellular functions.

B. Attachment Factors:

These are glycoproteins that facilitate cell adhesion, which is particularly important at the beginning of culture when cells are sparse and have not yet conditioned the culture medium. Examples include:

- ✓ **Albumin:** Provides a surface for cell attachment.
- ✓ **Transferrin:** Facilitates iron transport, supporting cell growth.
- ✓ **Fibronectin:** Promotes cell adhesion to the substrate.

C. Protective Elements:

These include protease inhibitors that protect cells from proteolysis, thereby increasing cell viability and stability.

D. Nutritional Properties:

FBS is rich in trace elements and minerals such as Na^+ (sodium), K^+ (potassium), Zn^{2+} (zinc), and Fe^{2+} (iron), which are essential for cell metabolism and growth.

- **Note:** The addition of antibiotic mixtures (e.g., penicillin and streptomycin) is necessary to control bacterial and yeast growth, ensuring that cell culture is conducted in a completely sterile environment.

1.2.2 Advantages and Disadvantages of Using Fetal Bovine Serum (FBS):

Fetal Bovine Serum (FBS) is widely used in cell culture due to its ability to support cell growth and proliferation. However, it comes with both benefits and drawbacks. Below is a summary of its key advantages and disadvantages:

Table 2: Advantages and Disadvantages of Using Fetal Bovine Serum (FBS)

Advantages	Disadvantages
Growth Factors: Provides essential growth factors that promote cell proliferation.	**Undefined Composition:** The exact composition of FBS is not fully defined, leading to variability.
Hormones: Supplies hormones necessary for cell growth and differentiation.	**Batch Variability:** Significant variability between different batches of FBS.
Attachment Factors: Enhances cell adhesion, which is crucial for cell division and growth.	**Safety Concerns:** Even when collected and sterilized, FBS may contain viruses, toxins, or mycoplasmas.
Protective Role: Increases cell viability and stabilizes cellular products.	**High Protein Content:** Interferes with the purification of specific proteins (e.g., recombinant or therapeutic proteins).
Nutritional Properties: Contains metabolites, ions, trace elements, and lipids essential for cell growth.	**Supply Variability:** Inconsistent supply can lead to price fluctuations.
Buffering Capacity: Helps maintain a stable pH in the culture medium.	**Fibroblast Preference:** Promotes the growth of fibroblasts over other cell types in primary cultures.
Transport Proteins: Facilitates the transport of low molecular weight substances like iron and fatty acids.	**Ethical Concerns:** Ethical issues related to the collection of FBS from fetal calves.
Detoxifying Ability: Absorbs and neutralizes toxins in the culture medium.	**Regulatory Challenges:** Strict regulatory requirements for the use of biological products.

1.3 Defined Media or Serum-Free Synthetic Media:

Due to the low reproducibility of certain experimental results, researchers have shifted toward developing serum-free media enriched with rigorously controlled factors. These media are referred to as defined media. Various defined components are added at specific concentrations to a rich synthetic basal medium (e.g., HAM F12 or MCDB 104). The addition of these substances varies depending on the requirements of the cell type being cultured.

These substances may include:

- ✓ **Hormones**
- ✓ **Growth Factors**
- ✓ **Attachment Factors**
- ✓ **Transport Proteins**
- ✓ **Antioxidants**
- ✓ **Lipid Substances**
- ✓ **Miscellaneous Factors**

1.3.1 Hormones:

Insulin: Used at concentrations of 5 to 10 µg/mL, insulin is an important substance for certain cell lines. However, it is not very stable in serum-free media, so its role is often limited to the early stages of culture. It may become unnecessary once cells are in full growth phase.

1.3.2 Growth Factors:

Growth factors are classified as paracrine or autocrine substances (if the mediator acts over a short distance by diffusing toward its target, it is paracrine; if the target is a cell of the same type, it is autocrine). More generally, they are referred to as local chemical mediators. Their action on cells is triggered by the formation of a specific receptor complex, resulting in:

- ✓ An increase in cell size,
- ✓ An increase in cell population,
- ✓ Induction or inhibition of a differentiation step.

Growth factors are polypeptides, most of which are now produced in sufficient quantities. However, serum-free media containing these factors can be very expensive. Such media are primarily used for research and rarely for large-scale production.

Examples of growth factors include:

- ✓ **EGF (Epidermal Growth Factor):** Promotes the growth of epithelial cells.
- ✓ **FGF (Fibroblast Growth Factor):** Stimulates fibroblast proliferation and differentiation.
- ✓ **NGF (Nerve Growth Factor):** Supports the growth and survival of neurons.
- ✓ **PDGF (Platelet-Derived Growth Factor):** Promotes the growth of endothelial cells derived from platelets.
- ✓ **TGF (Transforming Growth Factor):** Regulates cell growth, differentiation, and apoptosis.
- ✓ **Interferons:** Involved in immune response and cell signaling.
- ✓ **Interleukins:** Play a role in immune system regulation and cell communication.

1.3.3 Attachment Factors:

Surfaces coated with protein substrates provide an ideal environment for culturing cells that are difficult to adhere. These substrates also promote the differentiation of cultured cells. Some attachment factors can be introduced into the culture medium in solution, such as Poly-L-lysine and Collagen. These factors enhance cell adhesion and spreading, which are critical for cell survival and function in vitro.

1.3.4 Transport Proteins:

- ✓ **Bovine Serum Albumin (BSA):**

BSA can be used at a maximum concentration of 5 g/L. It serves multiple functions, including acting as a carrier for lipophilic substances (e.g., fatty acids, trace elements, hormones, and fat-soluble vitamins) and detoxifying the medium (e.g., transporting H_2O_2 and binding toxins). Its versatility makes it a key component in serum-free media.

- ✓ **Transferrin:**

Used at concentrations of 1 to 100 mg/L, transferrin primarily transports iron, which is essential for cell metabolism. It also helps detoxify other metals, ensuring a safe environment for cell growth.

1.3.5 Antioxidants:

Antioxidants play a crucial role in serum-free cultures. They neutralize peroxides generated during cell growth, a function typically performed by serum. Common antioxidants include glutathione, vitamin E, and ascorbic acid, which protect cells from oxidative stress and improve cell viability.

1.3.6 Lipid substances:

Lipid substances are essential for cells as they provide precursors for the synthesis of prostaglandins, which are necessary for certain specialized cells. These lipids are incorporated into cellular membranes after potential modifications. Ethanolamine (10-20 µM) is a required component in the formulation of most serum-free media, supporting membrane integrity and cell signaling.

1.3.7 Miscellaneous Factors:

- Trace Metals: For example, selenium, a component of the enzyme glutathione peroxidase. This enzyme plays a critical role in breaking down toxic peroxides metabolized by cells during culture. Selenium also supports antioxidant defense mechanisms, enhancing cell viability.

- Ascorbic Acid (Vitamin C): Vitamin C is essential for maintaining the redox potential within the culture medium. It acts as an antioxidant, protecting cells from oxidative stress and supporting collagen synthesis in certain cell types.

- Nucleic Acid Precursors: Such as thymine, uridine, and adenosine, which are necessary for DNA and RNA synthesis. These precursors support cell proliferation and are particularly important for rapidly dividing cells.

1.3.8 Undefined Substances:

Substances like peptones are used to enhance both serum-containing and serum-free media. They provide a mixture of amino acids, fatty acids, salts, oligopeptides, and other nutrients. The advantage of using peptones is that serum-free media containing these substances can be more easily utilized compared to media without such additions. Peptones are particularly useful in industrial applications where cost-effectiveness and scalability are important.

1.3.9 Advantages and Disadvantages of Defined (Serum-Free) Media:

❖ Advantages of Defined Media:

Optimized Cell Growth and Productivity: Serum-free media can be tailored to specific cell types, leading to improved growth rates and higher productivity.

Reproducibility: The absence of serum eliminates batch-to-batch variability, ensuring consistent experimental results.

Cost Reduction: In some cases, the cost of serum-free media can be lower, especially if the addition of expensive growth factors is minimized.

Reduced Risk of Contamination: Serum-free media eliminate the risk of introducing contaminants such as viruses, mycoplasma, or prions that may be present in serum.

❖ Disadvantages of Defined Media:

Development and Optimization Challenges: Creating and optimizing serum-free media for different cell types can be complex and time-consuming. Each cell type may require a unique formulation to support its growth and function.

High Initial Costs: The development and testing of serum-free media can be expensive, particularly when specialized growth factors or supplements are required.

Limited Applicability: Some cell types, particularly primary cells, may not adapt well to serum-free conditions, requiring additional modifications or supplements.

2. Standard Protocol for Media Preparation:

Preparing cell culture media requires careful attention to detail to ensure optimal growth conditions and sterility. Below is a step-by-step guide to creating a reliable and effective culture medium for your cells.

✓ **Retrieve Cell Culture-Specific Dishes and Flasks:**

Use cell culture-treated dishes and flasks that have been sterilized using X-rays or other sterilization methods. These containers are designed to promote cell adhesion and growth while maintaining sterility.

✓ **Obtain a Synthetic Culture Medium:**

Choose a synthetic culture medium, which primarily consists of a physiological liquid to prevent cell lysis. The medium provides the necessary nutrients and environment for cell survival and proliferation.

✓ **Use DMEM (Dulbecco's Modified Eagle's Medium):**

DMEM is the most commonly used culture medium due to its versatility and ability to support a wide range of cell types. It contains a balanced mixture of salts, glucose, and amino acids.

✓ **Add Vitamins, Glucose, and Essential Amino Acids:**

Supplement the medium with vitamins (e.g., B vitamins), glucose (as an energy source), and essential amino acids (e.g., L-glutamine) to meet the metabolic needs of the cells.

✓ **Include a pH Indicator (e.g., Phenol Red):**

Add phenol red to monitor the pH of the medium. The medium turns red at physiological pH (around 7.4). A loss of color indicates a pH shift, signaling the need to replace the medium to prevent cell death.

✓ **Add Fetal Bovine Serum (FBS):**

FBS provides essential growth factors, hormones, and nutrients necessary for optimal cell growth and development. It is typically added at a concentration of 5-20%, depending on the cell type.

✓ **Incorporate Antibiotics (e.g., Penicillin and Streptomycin):**

Add antibiotics to prevent bacterial or fungal contamination, especially if aseptic techniques are not perfectly maintained. A common concentration is 100 U/mL penicillin and 100 µg/mL streptomycin.

✓ **Stabilize the pH with a Buffer (e.g., HEPES):**

Add HEPES buffer to maintain a stable pH in the medium, particularly if the culture is exposed to air or frequent changes in CO_2 levels.

✓ **Inoculate the Cell Line and Incubate:**

Once the medium is prepared, introduce the cell line into the medium. Incubate the culture at **37°C** under **5% CO_2**, which mimics physiological conditions.

✓ **Set the Incubator Parameters:**

Ensure the incubator is set to maintain the required temperature and CO_2 levels. Avoid frequently opening the incubator to prevent contamination and CO_2 loss, which can affect pH and cell growth.

❖ **Notes:**

Sterility: Always work in a laminar flow hood to maintain sterility during media preparation and cell handling.

Quality Control: Regularly check the pH, osmolarity, and sterility of the medium to ensure optimal cell growth conditions.

Customization: Adjust the medium composition based on the specific requirements of the cell type being cultured. For example, some cells may require additional growth factors or supplements.

This protocol ensures the preparation of a high-quality culture medium that supports cell growth, minimizes contamination risks, and maintains reproducible experimental conditions.

Chapter 4:
From Isolation to Characterization of Cultured Cells

1. Cell Isolation:

The process of obtaining cells varies significantly depending on whether they originate from unicellular or multicellular organisms. For unicellular organisms (e.g., yeast, bacteria), cells are directly collected from their natural environments and transferred into an appropriate culture medium. For example, bacterial cell culture is relatively straightforward. However, isolating cells from multicellular organisms involves a more complex and meticulous process.

1.1 Obtaining Cells:

Multicellular organisms consist of two main types of cells, each requiring different isolation techniques:

A. Free or Circulating Cells:

These cells exist in suspension and are not attached to any surface or tissue. They live in cohesion with each other and form tissues such as blood, bone marrow, or amniotic fluid.

- ✓ **Isolation Method:** Circulating cells are typically isolated through a process called **Ficoll centrifugation**, which separates cells based on their density. For example, peripheral blood mononuclear cells (PBMCs) or stem cells from bone marrow can be isolated using this technique.
- ✓ **Advantages:** This method is relatively simple and yields a high purity of target cells.
- ✓ **Applications:** Commonly used in immunology, hematology, and stem cell research.

B. Cells Organized in Tissues or Organs:

These are adherent cells that are tightly bound together in tissues or organs. To isolate them, mechanical methods (e.g., dissection or grinding) or enzymatic methods (e.g., proteolytic enzymes like trypsin or collagenase) are used to break down the extracellular matrix. Once isolated, these cells can be placed in primary culture and later subcultured into secondary culture (Figure 14).

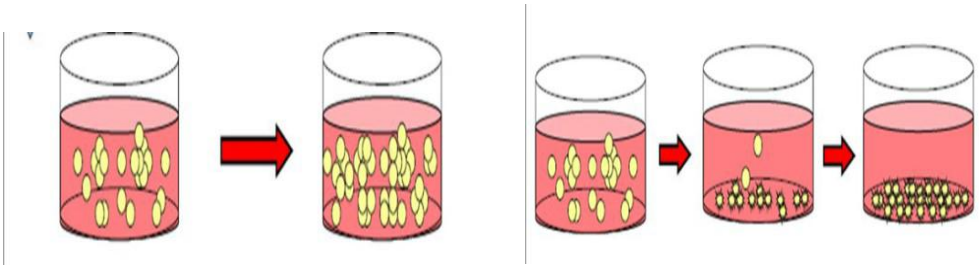


Figure 14 : Suspension culture and adherent culture.

1.2 Techniques for Tissue and Cell Isolation:

A. Dissection Methods:

These are among the oldest techniques used in tissue culture and were instrumental in enabling early researchers to obtain the first cells for in vitro culture.

- Carrel's Method:

This method involves taking a small piece of tissue, cutting it into a very small rectangle, and placing it on a mixture of two drops of chicken plasma and two drops of 50% embryonic extract. After incubating for 24 hours in an incubator, the first cells begin to migrate out of the explant.

- Dissection Method:

For example, in skin tissue isolation, the tissue is cut into fragments of approximately 1 to 4 mm^3. These fragments are further reduced using forceps and then placed in a culture flask containing a nutrient medium. Cells migrate from the fragments and begin to multiply. This technique is also known as the explant method.

- Mechanical Method:

This technique is suitable for soft tissues such as the thymus or spleen. The tissue is rubbed against a mesh, filtered, centrifuged, and then placed in culture. Alternatively, tissues can be dissociated by repeatedly pipetting them using a glass pipette.

B. Enzymatic Methods:

Enzymatic methods use proteolytic enzymes to digest the protein matrix surrounding cells, allowing their release. Commonly used enzymes include trypsin, collagenase, hyaluronidase, elastase, dispase, and papain. It is crucial to adjust the enzyme concentration based on the tissue type to achieve optimal dissociation without damaging cell membranes. For example, collagenase is used for collagen-rich tissues.

- **Example: Use of Trypsin:**

Primary tissue fragments are cut into small pieces of 2 to 3 mm. A trypsin solution is added at a ratio of 1 g of tissue per 10 mL of solution. The suspension is gently mixed using a magnetic stirrer at 37°C for 30 minutes to avoid cell damage. The supernatant is then collected and centrifuged at 600 g for 5 minutes to recover the cells that have detached due to enzymatic action. The cells are found in the pellet **.**

1.3 Advantages and Disadvantages of Techniques:

The choice of cell isolation technique depends on the nature of the tissue, the type of cells required, and the specific goals of the experiment. Two commonly used methods are the dissection method and the enzymatic method, each with its own advantages and limitations. The dissection method is often preferred for small tissue samples, while the enzymatic method is favored for its speed and efficiency. Below is a comparison of these two techniques to help researchers select the most appropriate approach for their work.

Table 1: Comparison Between Dissection Method and Enzymatic Method

Method	Advantages	Disadvantages
Dissection Method	- Often used when the tissue to be cultured is very small.	- The time required to obtain confluent cell layers is relatively long (about 30 days).
Enzymatic Method	- Much faster with a high yield.	- Cells with fragile membranes may be damaged by this method.

2. Culture Conditions:

A happy environment allows cells to do more than just survive in culture. Generally, this means an environment that, at a minimum, enables cells to increase in number through cell division. To achieve this environment, it is essential to provide cells with specific conditions,

adhering to certain physicochemical parameters necessary for cell culture, typically maintained using an incubator. These physicochemical parameters include: oxygen/CO_2 ratio, temperature, pH, and osmolarity. Additionally, cell culture requires an appropriate culture medium and strict sterility conditions.

2.1 Critical Parameters

2.1.1 Oxygen:

The optimal oxygen level varies depending on the cell type. Most cells are cultured under normoxic conditions, meaning an ambient oxygen level of 21%. Hyperoxia (oxygen levels above 50%) becomes toxic to cells, while hypoxia (low oxygen levels) can be beneficial in some cases, particularly for embryonic cells, which show increased proliferative capacity under low oxygen tension (5%).

2.1.2 CO_2:

The CO_2 concentration is particularly important when using the bicarbonate buffer system. It is maintained at 5% of the ambient air. CO_2 levels also play a role in cell proliferation and the synthesis of purine and pyrimidine bases.

2.1.3 Temperature:

Cells must be maintained at the temperature corresponding to the organism from which they are derived. The optimal temperature for mammalian cell growth is between 35.1°C and 37.1°C, requiring cell cultures to be kept in an incubator at around 37°C. Some cells can grow and proliferate at lower temperatures, but high temperatures are generally poorly tolerated, with a significant increase in cell mortality above 40°C.

2.1.4 Osmolarity:

The atmosphere must be saturated with water vapor to limit evaporation. Therefore, the incubator atmosphere should be saturated with water vapor, often achieved by flooding the incubator floor. Evaporation can increase the osmolarity of the culture medium, leading to higher salt concentrations and causing cell lysis due to water being drawn out of the cells. The

osmolarity of the medium should be maintained around 270 milliosmoles for most cell cultures, with water vapor saturation around 84-85%.

2.1.5 pH:

The optimal pH varies depending on the type of cells being cultured and the organism from which they are derived. For mammalian cells, the pH should be within the physiological range of 7.2 to 7.4. pH regulation is mandatory for mammalian cells and can be achieved through two systems:

→ Addition of a buffering agent to the culture medium, such as HEPES.
→ Addition of sodium bicarbonate ($NaHCO_3$) to the medium and CO_2 gas to the incubator atmosphere.

❖ **pH Changes:**

- ✓ **Acidification:** Spontaneous acidification of the culture medium can occur due to CO_2 release from cellular metabolism when the cell population becomes too large.
- ✓ **Alkalization:** Alkalization can occur during cell death, caused by the release of alkaline proteins during cell lysis.
- ✓ **Contamination Effects:** Fungal contamination can cause medium alkalization, while bacterial contamination leads to acidification.

2.2 pH Regulation:

pH regulation is a critical aspect of cell culture, as it directly impacts cell viability, growth, and function. One of the most commonly used tools for monitoring pH in real-time is the **phenol red pH indicator**. This indicator provides a visual representation of the pH level in the culture medium, allowing researchers to assess the state of the culture at any given moment.

❖ **Phenol Red Indicator:**

Phenol red is a pH-sensitive dye that changes color based on the acidity or alkalinity of the medium. It is widely used in cell culture to monitor pH fluctuations.

- ✓ **Optimal pH (7.2–7.4):** A well-regulated medium will appear **orange-red**, indicating a physiological pH suitable for most mammalian cells.
- ✓ **Acidic Conditions (pH < 7.0):** The medium turns **yellow**, signaling acidification, which can occur due to excessive cell metabolism or bacterial contamination.
- ✓ **Alkaline Conditions (pH > 7.6):** The medium turns **purple-red**, indicating alkalization, often caused by cell death or fungal contamination.

Maintaining the correct pH is crucial for ensuring cell health, as it directly impacts cell survival and proliferation. Sudden changes in pH can serve as an early indicator of microbial contamination, prompting researchers to take immediate corrective actions to prevent further issues. Additionally, pH shifts can reflect changes in cellular metabolism, such as increased CO_2 production during periods of high cell density. By closely monitoring pH, researchers can maintain optimal culture conditions, ensuring consistent and reliable experimental outcomes.

3. Cell Characterization:

The characterization of cells in cell culture primarily depends on the type of cells:

- ✓ Normal cells: These have a limited number of subcultivations (Hayflick limit).
- ✓ Transformed cells (or potentially embryonic cells): These can undergo an unlimited number of passages, resulting in a continuous cell line. The most commonly used cells in cell culture and medical virology are Vero cells and African green monkey kidney cells (Cercopithecus aethiops). These cells have a fibroblast-like (spindle-shaped) appearance and adhere quickly to glass or plastic supports due to the presence of Ca2+ or Mg2+ ions.
- ✓ Tumor cells: Notably, HeLa cells (derived from a uterine tumor) and KB cells (derived from a human oral carcinoma).
- ✓ Embryonic cells: Notably, MRC-5 cells (derived from human fetal lung) and 3T3 cells (derived from mouse embryos).

3.1 Hayflick Limit:

The Hayflick limit was discovered by Leonard Hayflick in 1965. He observed that dividing cells (undergoing mitosis) in a cell culture could only divide about 50 times before dying. As

cells approached this limit, they exhibited signs of senescence. This limit varies depending on the cell type and, even more so, on the organism type. For humans, the limit is around 50 divisions. This limit has been linked to the shortening of telomeres.

Consequently, normal cells typically cannot be maintained in culture indefinitely, primarily due to their limited number of divisions (~50 times, as per the Hayflick limit). The life and death of normal cells are programmed (Figure 16).

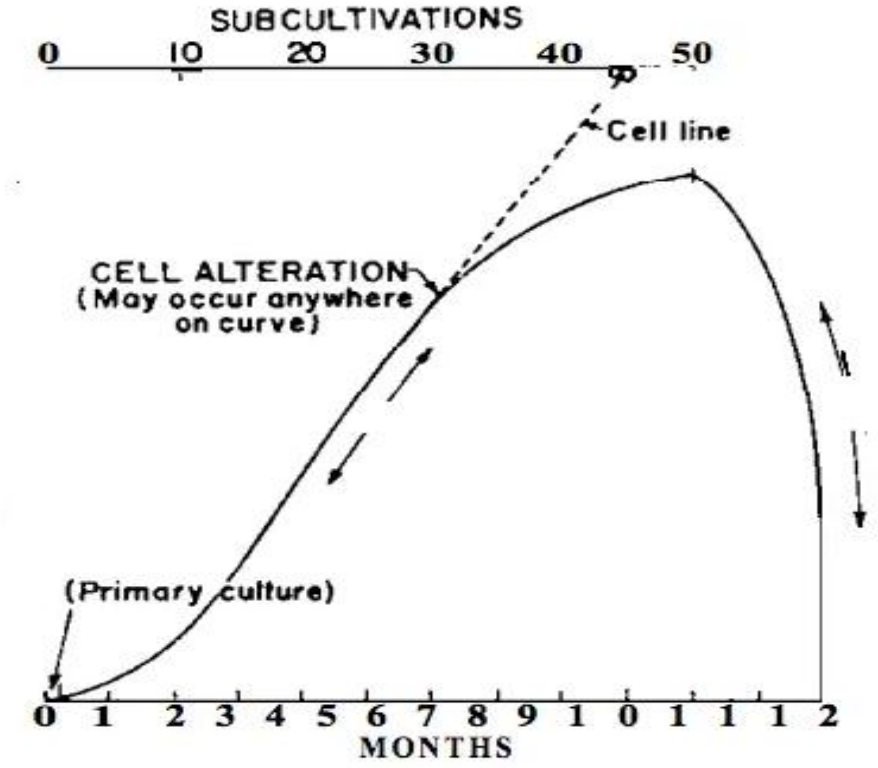


Figure 16 : Hayflick limit.

3.2 Cellular Immortality

Transformed cells are cells with an unlimited division capacity (referred to as immortality in culture) and are known as "cell lines." These lines can be either cancer cells (such as the rapidly developing HeLa cells from Henrietta Lacks), cells in the process of becoming cancerous, or healthy cells that have been artificially rendered "immortal."

- **Note:**

Cancers only become problematic if the cells that compose them have found a way to bypass the Hayflick limit. Such cells are called "immortal cells." These immortal cells eventually die, but the population of immortalized cells is not limited in terms of the number of cell divisions that can occur within it.

3.3 Characterization of Cells in Continuous Culture (Transformed Cell Lines):

In continuous culture or immortalized/transformed cell lines:

The rate of multiplication does not decrease, allowing for an indefinite number of passages.

The cells constituting these cultures:

- ✓ Lose contact inhibition and grow in clusters or multilayers.
- ✓ Can often be cultured in suspension (lose their dependence on a substrate). Change morphology (become rounded).
- ✓ Adherent cells lose their need for anchorage and can be cultured in suspension.
- ✓ Immortality.
- ✓ Growth autonomy.
- ✓ Independence from growth factors.

3.4 Cell Growth: Normal Case

In cell culture, the growth rate of cells is not constant. Instead, it follows a curve that can be divided into four phases (Figure 17):

A. Adaptation Phase (Lag Phase):

There is practically no cell growth during this phase, as the cells adapt to their new environment and settle in.

B. Rapid Growth Phase (Exponential or Log Phase):

The cells divide rapidly, consuming most of the nutrients available in the culture medium.

C. Stationary Phase (Plateau Phase):

The number of cells remains constant because the rate of cell death equals the rate of new cell production. This is due to nutrient depletion, waste accumulation, and lack of available space.

D. Decline Phase:

Nutrients and space (contact inhibition) become too scarce to sustain the maximum number of cells. The cell count decreases, and the proliferation rate slows due to senescence (aging) = programmed cell death in non-transformed cells.

Case of Transformed Cells:

The rate of multiplication does not decrease: cells are immortalized.

❖ **Note:**

The maximum number of cells is reached at the end of the rapid growth phase. When the stationary phase is reached, it may be useful to stop the culture and preserve it for analysis or future use. Cultures can be frozen for storage.

It is also during this stationary phase that cells should be subcultured (transferred) into a new culture medium, as the nutrients in the initial medium are becoming depleted, which would otherwise trigger the decline phase.

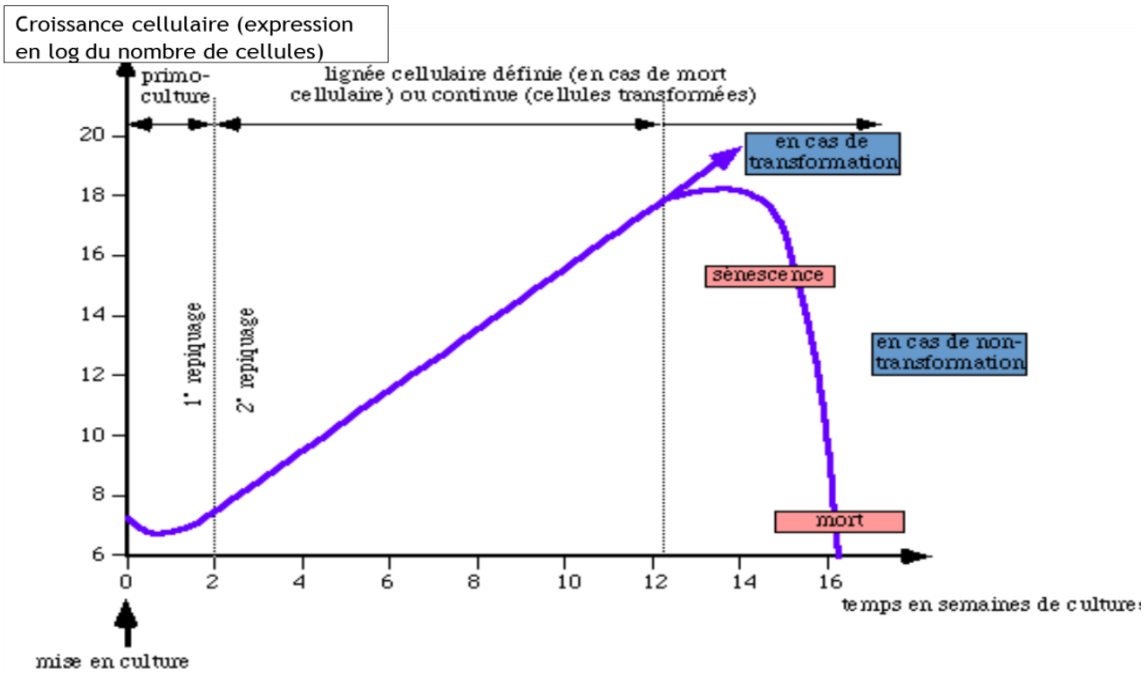


Figure 17: Growth of cells as a function of time in cell culture.

3.5 Cell Growth: Transformed Lines (e.g., HeLa)

Three distinct phases are identified: the adaptation phase (Lag), the exponential phase (Log), and the stationary phase (Plateau) (Figure 17).

A. Lag Phase: Period of adaptation to the environment. The cell rebuilds its cytoskeleton, attaches to the substrate, and spreads out. DNA and protein synthesis occur.

B. Log Phase: Exponential growth phase. The cells divide rapidly as they consume most of the nutrients available in the culture medium.

C. Plateau Phase: The rate of cell multiplication does not decrease, allowing for an indefinite number of passages.

Transformed cells reach high plateaus. Having lost their dependence on a substrate, they can often be cultured in suspension.

However, certain cells, such as skin embryo fibroblasts, exhibit contact inhibition but continue to grow by organizing into overlapping layers.

- **Note:**

When cells are placed in a culture medium, a selection occurs between viable cells and dead cells (in the case of adherent cells, viable cells attach to the substrate while dead cells remain in the culture medium).

Additionally, there is competition among viable cells. Those that proliferate the fastest dominate the culture until other cell types disappear (e.g., in the case of bacterial contamination). Changes in the culture over time are observed.

4. Mitogenic Factors :

4.1 Definition and Role of Growth Factors:

A mitogen is an agent that promotes mitosis and cell division. It is the opposite of what anticancer treatments, also called anti-mitotic agents, aim to achieve. Mitogens are most often substances of plant origin, such as phytohemagglutinin, extracted from red kidney beans.

Mitogenic factors are growth factors that act in cell division by stimulating the cell cycle. They can stimulate the proliferation of many types of cells or be specific (for example, erythropoietin "EPO": a factor stimulating the formation of erythrocytes (Figure 18)).

The term "mitogenic factors" encompasses several proteins or glycoproteins that are unrelated to each other but all have an effect on the growth of a specific cell type.

The term "growth" refers to both the multiplication of cells and the enlargement of an organism.

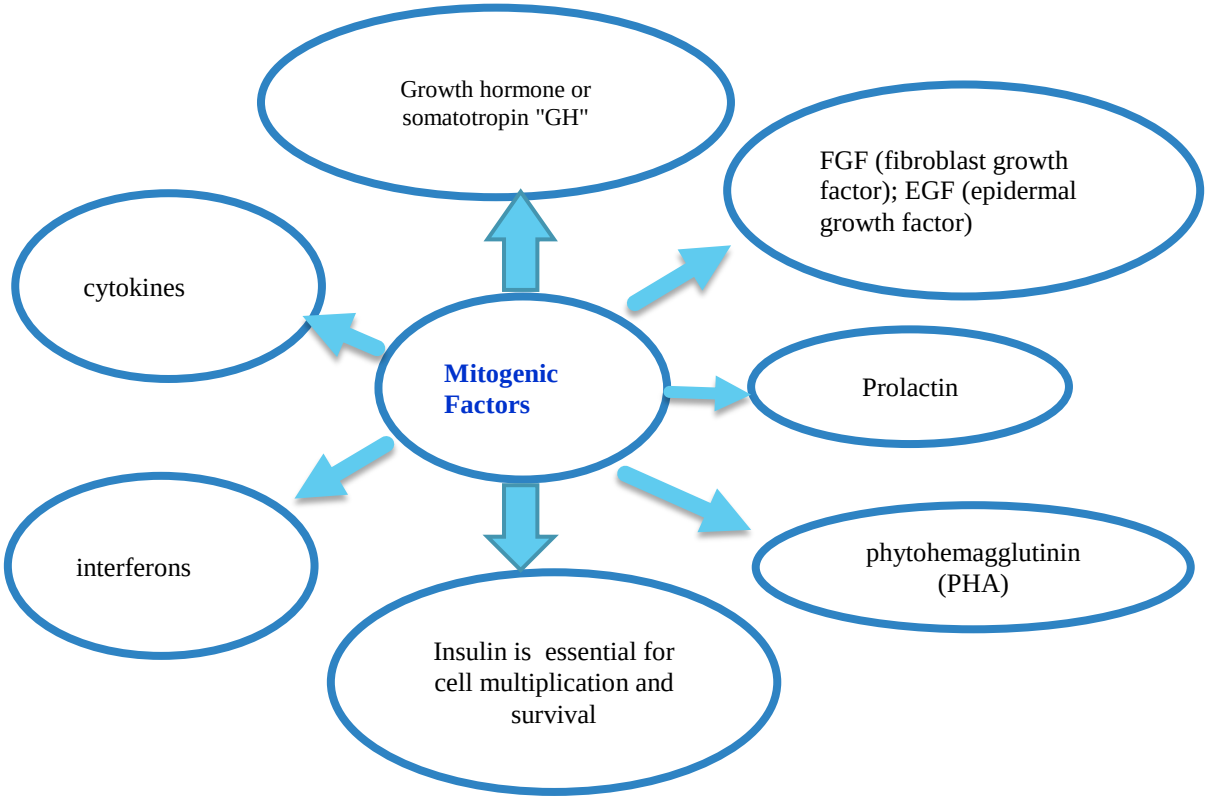


Figure 18: Mitogenic factors**.**

4.2 Growth Factors for Cell Culture:

A growth factor is a molecule that meets the following criteria:
The majority of its structure is polypeptide.
Its interaction with the membrane of the target cell initiates a cellular response, which is

triggered only by the formation of a specific complex with a receptor. The formation of the growth factor/receptor complex specifically results in:

- ✓ A hypertrophic response (increase in cell size),
- ✓ A hyperplastic response (increase in cell population),
- ✓ A response that induces or blocks a differentiation step.
- ✓ The growth factor and its receptor are removed from the cell by endocytosis.

4.3 Main Growth Factors:

The main growth factors are:

Epidermal Growth Factor (EGF): Acts on epidermal cells and plays an important role in embryonic development.
Insulin-Like Growth Factor (ILGF): Exhibits insulin-like activity that is not suppressible by anti-insulin antibodies.
Nerve Growth Factor (NGF): Enhances the survival and differentiation of glial cells.
Platelet-Derived Growth Factor (PDGF): A growth factor derived from platelets, necessary for fibroblast division. It is the primary mitogen for mesenchymal cells.
Transforming Growth Factor (TGF): TGF encompasses a large number of different growth factors secreted by tumor cells or cells transformed in vitro. For example, TGF-β (transforming growth factor beta) stimulates the proliferation of certain cells, inhibits it in others, and controls the differentiation of specific cells.
Fibroblast Growth Factor (FGF): A large family of factors that stimulate the growth of cells of mesodermal and ectodermal origin in vitro; these factors exhibit high affinity for heparin.
Erythropoietin (EPO): Promotes the proliferation and differentiation of red blood cell precursors.
Interleukin-2 (IL-2): Stimulates the proliferation of activated T lymphocytes.
Insulin-Like Growth Factor I (IGF-I): Stimulates cell metabolism and proliferation in combination with other growth factors.

- ❖ **Note:** Other molecules are discovered each year, along with new properties or characteristics for each of these factors.

❖ **Phytohemagglutinin (PHA):**

Phytohemagglutinin is a lectin found in plants, particularly in legumes such as beans. It is used as a mitogen to trigger cell division in T lymphocytes.

4.4 Applications:

Mitogens are useful in oncology for analyzing tumor chromosomes: they promote cell divisions before blocking them to examine individualized chromosomes during mitosis. In immunotherapy, they stimulate the proliferation of immunocompetent cells taken from the patient, potentially from within the tumor, to produce large quantities of cytotoxic cells in cell culture. These cells are then reinjected into the patient for treatment.

5. Contamination in Cell Culture:

Contamination is a natural problem in primary cultures, so antibiotics must be added, and samples must be decontaminated, which induces additional stress on the cells. To avoid contamination, it is essential to adopt good sterile techniques, handle cultures with care, and establish strict protocols in culture rooms. Indeed, if these rules are not followed, contamination can quickly spread to multiple cell lines, or even the entire stock of cell lines.

Whether visible or not, destructive or not, contaminants affect growth, alter cellular characteristics and functions, and impact the quality of results. Sometimes, certain agents are not toxic individually but can become toxic either due to their high concentration or when combined with another agent. Additionally, some cell types are more sensitive than others. Here are four good reasons to address contamination: Loss of money and time.

- ✓ Erroneous or inappropriate experimental results.
- ✓ Loss of important products.
- ✓ Personal embarrassment (damage to reputation due to erroneous results).

5.1 Sources of Contamination:

There are several possible sources of contamination:

- ✓ Improper sterilization of labware.
- ✓ Dust and spores due to airborne particles in the room and movement of people.

- ✓ Incubators not cleaned regularly.
- ✓ Refrigerators not cleaned regularly.
- ✓ Laminar flow hoods not cleaned or filters not checked.
- ✓ Importation of contaminated cell lines from other laboratories.
- ✓ Negligence and poor cell culture practices.
- ✓ Human error.

5.2 Types of Contamination:

5.2.1 Biological Contaminants:

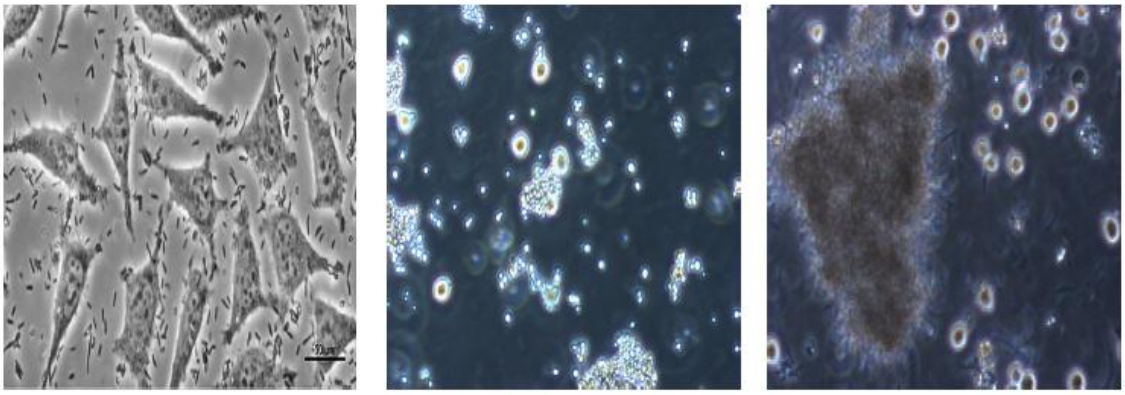
A. Bacteria - Yeast - Fungi:

Contamination by bacteria, yeast, and fungi can often be visible to the naked eye. The rapid growth of these microorganisms (especially in the absence of antibiotics) allows for their detection in a short time: turbidity, presence or absence of cloudiness, changes in pH, and cell death. Verification under a microscope is mandatory (Figure 19).

Bacteria: Cloudy medium, decrease in pH.

Yeast: Cloudy medium, little change in pH.

Fungi: Clear medium, increase in pH.



A b c

Figure 19: Microbial contamination in mammalian cell cultures. a: bacterial contamination; b: yeast contamination; and c: fungal contamination.

Bacteria:

Bacterial contamination is easily detected through visual inspection. Infected cultures typically appear cloudy (i.e., turbid), sometimes with a thin film on the surface. Bacteria are

mobile, have defined shapes, grow rapidly, and quickly cloud and acidify the culture medium (a sudden drop in pH). Under a low-power microscope, bacteria appear as tiny, granular particles moving between cells, and high-power microscopy can resolve the shapes of individual bacteria (Figure 20).

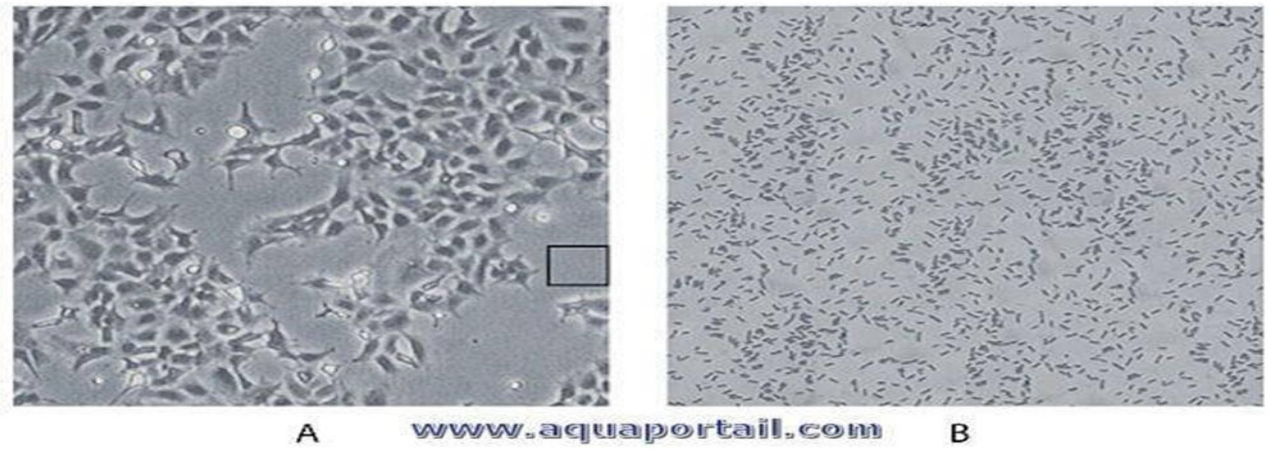


Figure 20 : Bacterial contamination by *Escherichia coli*..

The images above show simulated phase-contrast biological contamination of adherent 293 cells by Escherichia coli.

The spaces between the adherent cells reveal tiny granules under low-power microscopy, but it is not easy to distinguish individual bacteria (Part A). Additionally, an enlargement of the area marked by the black square shows individual E. coli cells, which are typically rod-shaped and measure about 2 µm in length and 0.5 µm in diameter. Each side of the black square in Part A is 100 µm.

- **Yeasts, Molds:**

Yeasts, like bacteria, do not adhere to cells and float in suspension. However, there are various types of yeasts with different characteristics. Their growth is slow, and the formation of spores, spread by ventilation inside the incubator, can cause issues for users.

A. Viruses:

Viruses are too small to be detectable. Many cause a cytopathic effect (cell death). However, caution is required with primary human cultures (HIV, hepatitis B, Epstein-Barr, etc.).

- **Source:** Bovine serum may contain viruses.

B. Protozoa:

Most are unicellular, such as amoebas. Some can form spores.

Some have a cytotoxic effect (cells destroyed in less than 10 days).

- **Source:** Dust, dirt, air, and occasionally tissues.

C. Mycoplasmas:

Mycoplasmas are the most devastating and widespread contaminants. They are insidious and detectable only when cell death occurs. They are often referred to as the "cancer of cells." They can appear as filaments or cocci. No visible signs are observed when examining the cells, except in cases where they die. Over 20% of cell cultures are infected with mycoplasmas.

The effects can be long-term, as they have the ability to affect their host cells in most of their functions:

They can alter cell function, attachment, membranes, and propagation, and can also cause slowed growth, changes in morphology, chromosomal aberrations, and alterations in certain metabolic processes (amino acids, nucleic acids). To effectively detect mycoplasmas in a cell culture, it is recommended to use an indirect test, such as DNA staining with a fluorochrome.

This is a simple and relatively quick test that involves staining DNA with a fluorescent dye. When the stained and fixed cells are examined under a UV microscope equipped with the appropriate filter set, the DNA produces bright fluorescence. This test not only detects mycoplasmas but also other microbial contaminants.

Detecting mycoplasma contamination requires specific tests (e.g., using a fluorochrome and verification under a fluorescence microscope) (Figure 21).

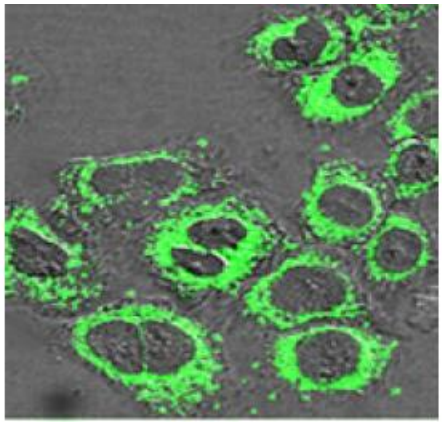


Figure 21 : Native melanoma cells infected with mycoplasma.

5.2.2 Chemical Contaminants:

Chemical contaminants can be a source of variability in results. This type of contamination can be caused by:

- ✓ Metal ions, endotoxins, and other impurities in the medium, serum, or water.
- ✓ Quality of disposable plastics (petri dishes, tubes, bottles).
- ✓ Free radicals and hydrogen peroxide (H2O2) present in the medium.
- ✓ Residue deposits on glassware or pipettes caused by detergent remnants or residue from aluminum foil.
- ✓ Residues of germicides or pesticides used when disinfecting incubators, countertops, or other instruments.
- ✓ Impurities in the CO2 supply of the incubator.
- ✓ The CO2 incubator also plays a significant role, as it provides ideal growth conditions for cell cultures but also for many unwanted microbes.
- ✓ Therefore, it is preferable to use an incubator equipped with multiple functions to prevent contamination.

5.2.3 Cross-Contamination:

Cross-contamination occurs due to the presence of multiple cell lines simultaneously under the hood. The most common example of this is undoubtedly HeLa cells.

Researchers have shown that approximately 25% of human cell lines are contaminated by HeLa-type cells.

To avoid this type of contamination, it is necessary to:

- ✓ Thoroughly disinfect the hood between each cell type. Ideally, a 20-minute wait is recommended.
- ✓ Handle only one cell line at a time under the hood.
- ✓ Never reinsert a used pipette into a bottle of medium or trypsin.
- ✓ Add the medium to the dishes first, then the cells, and close the culture dishes.
- ✓ Avoid opening different bottles and flasks of different cell lines simultaneously under the hood.

5.3 Treatment of Contamination:

Examine cultures under the microscope as soon as you handle them to check for contamination.

- ✓ Test for mycoplasma if contamination is suspected.
- ✓ If contamination is suspected, clean the hood and incubator.
- ✓ If contamination is confirmed, discard the contaminated cells (autoclave them and do not throw them in the trash), soak the used medium in bleach overnight, and discard the trypsin.
- ✓ Do not attempt to decontaminate; eliminate the culture.
- ✓ Attempt decontamination only if the cells are irreplaceable or in extreme situations.
- ✓ Restart cultures from frozen cell stocks.

Emergency Treatment:

Bacteria: Use various antibiotics.
Yeasts and fungi: Use antifungals.
Mycoplasmas: Use anti-mycoplasma agents.

5.4 Precautions to Prevent Contamination:

A. Operating Techniques:

- ✓ If reagents are sterile, the atmosphere is clean and dust-free, and equipment is clean, contamination depends on the handler.

- ✓ Handle one cell line at a time under the hood.
- ✓ Do not open different bottles and flasks of different cell lines simultaneously under the hood.
- ✓ Add the medium to the dishes first, then the cells, and close the culture dishes.

B. Environment:

- ✓ It should be as clean as possible.
- ✓ Avoid air disturbances caused by:

- ➢ Excessive movement of people.
- ➢ Frequent door openings.
- ➢ Storage of cardboard boxes.
- ➢ Refrigerators and centrifuges.

C. Laminar Flow Hoods:

- ✓ The work surface should be clean and organized to avoid disrupting the filtered airflow.
- ✓ Clean the bottom of the hood by removing the plates from the work surface once a week.
- ✓ Check the integrity of the hood filters once a year.

D. Incubators:

- ✓ Clean the incubator once a month or more frequently if it is opened often.
- ✓ Change the water in the humidity pan once or twice a month.
- ✓ If medium spills occur on the trays, replace the incubator trays, change the metal trays holding the culture dishes, and clean the humidity pan.
- ✓ If contaminated cultures are found in the incubator, remove them immediately by placing them in an autoclave bag, sealing it, and autoclaving. Then clean the incubator, including the fan.

E. Refrigerators:

Clean them twice a year.

F. Microscopes:

Clean microscope stages regularly with absorbent paper and 70% ethanol.

G. Water Baths:

Clean them twice a month.

H. Imported Cell Lines:

Pay special attention to primary cultures.

Handle cell lines imported from other laboratories with extreme caution and observe them frequently under the microscope to detect potential contamination as early as possible.

Chapter 5:
General Applications of Cell Culture

1. Fields of Application

Cell culture has become one of the major tools used in cellular and molecular biology. Some of the important fields where cell culture currently plays a significant role are briefly described below.

- ✓ **Study of cellular physiology mechanisms:** Control of the cell cycle, metabolism, regulation of gene expression, and study of cellular movements and junctions. Cell culture allows the study of eukaryotic cells and their interactions with their environment.
- ✓ **In human medicine:** Cell culture is used for grafts and autografts (blood stem cells), screening for genetic diseases (karyotype), and gene therapy.
- ✓ **Pharmaceutical industry:** This industry heavily relies on cell culture for pharmacological and toxicological studies of new drug molecules, production of therapeutic substances (growth hormones, insulin, interferons, cytokines, monoclonal antibodies), and vaccine production.

1.1 Model Systems

Cell cultures provide excellent model systems for studying:

- ✓ Basic cell biology and biochemistry.
- ✓ Interactions between cells and disease-causing agents.
- ✓ Effects of drugs on cells.
- ✓ Processes and triggers of aging.
- ✓ Nutritional studies.

1.2 Toxicity Testing

Cultured cells are widely used, either alone or in conjunction with animal testing, to study the effects of new drugs, cosmetics, and chemicals on the survival and growth of a wide variety of cell types. Liver- and kidney-derived cell cultures are particularly important.

1.3 Cancer Cell Research

Both normal and cancerous cells can be cultured, allowing for a detailed study of the fundamental differences between them. Additionally, it is possible to convert normal cultured cells into cancerous cells using chemicals, viruses, and radiation. This enables the study of the mechanisms leading to this transformation.
Cultured cancer cells also serve as test systems to determine drugs and methods suitable for selectively destroying certain types of cancers.

1.4 Virology

One of the earliest and most important uses of cell culture was the replication of viruses in cell cultures (instead of animals) for vaccine production. Cell cultures are also widely used in clinical virus detection and isolation, as well as in basic research to study how viruses develop and infect organisms.

1.5 The Cell as a Production Factory

While cultured cells can be used to produce many important products, three areas have proven particularly interesting. The first is the large-scale production of viruses for vaccine production, including vaccines for rabies, chickenpox, hepatitis B, and measles. The second is the large-scale production of genetically modified cells to produce proteins of medical or commercial value, such as monoclonal antibodies, insulin, and hormones. The third is the use of cells as replacements for tissues and organs. Artificial skin used for treating burns and ulcers is the first commercially available product. However, tests are ongoing for artificial organs like the pancreas, liver, and kidneys. A potential reserve of replacement cells and tissues may emerge from ongoing work with adult and embryonic stem cells, which have the potential to differentiate into various cell types. Learning to control the development of these cells offers hope for new treatment approaches for a wide range of diseases.

1.6 Genetic Engineering

The ability to transfect or reprogram cultured cells with new genetic material (DNA and genes) has provided a valuable tool for molecular biologists seeking to study the cellular effects of gene expression (new proteins). These techniques can also be used to produce these new proteins in large quantities in cultured cells for study. Insect cells are widely used as

miniature cell factories to express substantial amounts of proteins after being infected with genetically modified baculoviruses.

2. Advantages of Cell Culture:

2.1. Continuous and Homogeneous Cell Source:

Cell culture provides a consistent and uniform supply of cells that can be used across diverse fields, including biochemistry, medical research, and healthcare. This homogeneity ensures reproducibility in experiments and reduces variability in results.

2.2. Ease of Manipulation:

Unlike cells in vivo (within living organisms), cultured cells can be easily modified, observed, and controlled. Researchers can manipulate environmental conditions (e.g., nutrients, pH, temperature), introduce genetic changes, or test specific compounds directly on the cells.

2.3. Preservation of Cell Integrity:

Cell cultures can be cryopreserved (frozen) without compromising their ability to proliferate or altering their genetic makeup. This allows long-term storage of cell lines, ensuring their availability for future studies or applications.

2.4. Reduced Reliance on Animal Testing:

By using cell cultures, researchers minimize the need for laboratory animals in experiments. This not only lowers costs but also addresses ethical concerns by reducing the number of animals sacrificed for scientific purposes.

2.5.Cost-Effectiveness:

Cell culture systems are generally more economical than animal studies. They require fewer resources, less space, and eliminate the logistical and ethical challenges associated with maintaining live animal models.

2.6.Scalability:

Large quantities of cells can be produced under controlled conditions, enabling high-

throughput screening for drug development, toxicity testing, and industrial applications (e.g., vaccine production).

2.7. Ethical and Regulatory Benefits:

Cell cultures align with global efforts to promote alternatives to animal testing (the "3Rs" principle: Replacement, Reduction, Refinement). This approach is increasingly favored in regulatory frameworks for drug and cosmetic testing.

2.8. Versatility in Research:

Cultured cells serve as models for studying human diseases (e.g., cancer, viral infections), cellular pathways, and gene function in a simplified, isolated system. This accelerates discoveries in molecular biology and personalized medicine.

2.9. Applications in Biotechnology:

Cultured cells are "factories" for producing therapeutic proteins (e.g., insulin, monoclonal antibodies), vaccines, and engineered tissues, driving advancements in regenerative medicine and biopharmaceuticals.

2.10. Environmental Control:

Researchers can isolate specific variables (e.g., oxygen levels, growth factors) to study their effects on cells, which is challenging in complex living organisms.

Chapter 6:
Specific and Advanced Applications in Cell Culture

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1. Specialized Cell Cultures

Cell culture technologies have advanced dramatically, allowing researchers to maintain and analyze highly specialized cell types *in vitro*. These models are indispensable for replicating human physiology, dissecting disease mechanisms, and testing therapeutic strategies. This section examines four critical specialized cell cultures—hepatocytes, Langerhans islets, myositis cultures, and lymphocytes—detailing their biological relevance, experimental applications, and technical hurdles.

1.1 Hepatocytes

Hepatocytes, the principal functional cells of the liver, play a pivotal role in metabolism, drug detoxification, and regenerative medicine. Their capacity to synthesize plasma proteins, metabolize xenobiotics, and regulate glucose homeostasis makes them essential for *in vitro* toxicology and pharmacokinetic studies. In drug development, hepatocyte cultures are widely employed to predict hepatic clearance rates, metabolite profiles, and drug-drug interactions, reducing reliance on animal models. Primary human hepatocytes (PHHs) are particularly valuable for modeling diseases such as non-alcoholic fatty liver disease (NAFLD) and viral hepatitis, offering insights into pathogen-host interactions. Additionally, bioartificial liver devices, which integrate engineered hepatocytes into bioreactors, hold promise for temporary support in liver failure patients. However, maintaining hepatocyte functionality *in vitro* remains challenging due to rapid dedifferentiation and loss of metabolic activity. To address this, researchers have developed advanced 3D co-culture systems that incorporate non-parenchymal cells like Kupffer cells and stellate cells, alongside extracellular matrix (ECM) scaffolds, to better mimic the liver's microenvironment. Recent breakthroughs in induced pluripotent stem cell (iPSC) technology have further enabled the derivation of patient-specific hepatocytes, opening avenues for personalized drug testing and disease modeling.

1.2 Langerhans Islets

Langerhans islets, particularly their insulin-producing β-cells, are central to diabetes research and therapy. *In vitro* islet cultures provide a platform to study glucose homeostasis, autoimmune destruction in Type 1 diabetes, and β-cell dysfunction in Type 2 diabetes. These

cultures are critical for optimizing islet transplantation protocols, a therapeutic approach for severe diabetes, by improving viability and functional integration post-transplant. Drug screening applications focus on identifying compounds that enhance insulin secretion or protect β-cells from apoptosis, such as GLP-1 receptor agonists. Islet cultures also facilitate studies of autoimmune mechanisms; for example, co-culturing islets with T-cells reveals pathways involved in β-cell destruction. Despite their utility, islet cultures face significant technical challenges, including fragility, rapid loss of viability *ex vivo*, and limited nutrient diffusion in static culture systems. Innovations like microfluidic perfusion systems and oxygen-rich bioreactors have partially mitigated these issues. Moreover, human stem cell-derived islet organoids now replicate glucose-responsive insulin secretion with high fidelity, offering scalable models for diabetes research and reducing dependence on scarce donor tissues.

1.3 Myositis Cultures

Myositis cultures, derived from skeletal muscle biopsies or patient-specific cells, are vital for studying inflammatory myopathies such as dermatomyositis and polymyositis. These models dissect the complex interplay between muscle fiber degeneration, immune cell infiltration, and autoantibody production. By maintaining muscle cells in culture, researchers can identify pro-inflammatory cytokine signatures—such as elevated IFN-γ and TNF-α—that drive pathology. Therapeutic testing in these systems includes exposing myotubes to patient-derived autoantibodies to evaluate the efficacy of immunosuppressants like rituximab or JAK inhibitors. Proteomic analyses of cultured muscle cells further aid in discovering novel autoantigens and biomarkers for early diagnosis. However, replicating the inflammatory microenvironment *in vitro* requires precise combinations of cytokines, chemokines, and immune cell interactions, which are difficult to standardize. Patient heterogeneity adds another layer of complexity, as genetic and epigenetic variations influence disease progression and treatment responses. Cutting-edge approaches, such as muscle-on-a-chip platforms, integrate endothelial cells, immune cells, and mechanical stimulation to better emulate *in vivo* conditions, enhancing the translational relevance of these models.

1.4 Lymphocytes

Lymphocytes—including T cells, B cells, and natural killer (NK) cells—are indispensable for advancing immunology and immunotherapy research. Culturing these cells enables detailed

studies of adaptive immune responses, cancer immunotherapies, and autoimmune disorders. For instance, chimeric antigen receptor (CAR) T cells are expanded *in vitro* for adoptive cell therapies targeting hematologic malignancies. B-cell cultures, meanwhile, assess antigen-specific antibody production, aiding vaccine development and monoclonal antibody engineering. In autoimmune research, T-cell clones isolated from rheumatoid arthritis or lupus patients are cultured to investigate dysregulated signaling pathways and test targeted therapies. A key challenge lies in maintaining lymphocyte functionality during extended cultures, as these cells require precise activation signals—such as CD3/CD28 antibodies and interleukin-2 (IL-2)—to avoid anergy or apoptosis. Recent advances include CRISPR-Cas9 gene editing to enhance lymphocyte specificity and durability, as well as organoid co-cultures that incorporate tumor cells or stromal components to model immune-tumor interactions more accurately. These innovations are accelerating the preclinical development of next-generation immunotherapies.

2. Applications of Cell Cultures to Cancer Cells

Cell culture technologies have become indispensable in the study and management of cancer, offering a controlled platform to dissect tumor behavior, test therapies, and decode molecular pathways. By isolating cancer cells from their complex *in vivo* environments, researchers can systematically investigate their proliferation, survival, and interactions with drugs or immune cells. These *in vitro* systems bridge fundamental discoveries and clinical applications, enabling precision medicine approaches tailored to individual patients. From modeling tumor heterogeneity and drug resistance to engineering genetically modified cell lines, cell cultures empower scientists to explore cancer's vulnerabilities and adaptive strategies. This section delves into three critical domains: cancer cell models for basic research, therapeutic sensitivity testing, and the elucidation of molecular and genetic mechanisms driving malignancy.

2.1 Cancer Cell Models in Research

Cell culture systems have revolutionized cancer research by providing reproducible and controllable models to study tumor biology. Traditional two-dimensional (2D) monolayer cultures of cancer cell lines, such as HeLa or MCF-7, remain foundational tools for investigating proliferation, apoptosis, and metastasis. However, advancements in three-dimensional (3D) cultures, including spheroids and organoids, now better mimic the structural

and functional complexity of tumors *in vivo*. Primary cancer cell cultures derived directly from patient biopsies offer unique insights into tumor heterogeneity and personalized therapeutic responses. Additionally, co-culture systems incorporating stromal cells, immune cells, and extracellular matrix components enable researchers to dissect tumor-microenvironment interactions. These models are indispensable for unraveling the hallmarks of cancer, from sustained proliferative signaling to evasion of immune destruction.

2.2 Sensitivity Testing to Treatments

Cell cultures play a pivotal role in evaluating the efficacy of anticancer therapies. *In vitro* sensitivity assays, such as the MTT or clonogenic survival tests, allow rapid screening of chemotherapeutic agents, targeted therapies, and immunotherapies. For instance, patient-derived cancer cells can be exposed to a panel of drugs to identify the most effective treatment regimen, reducing the reliance on trial-and-error approaches in clinical settings. High-throughput screening (HTS) platforms further accelerate drug discovery by testing thousands of compounds against cancer cell libraries. Notably, organoid models are increasingly used to predict patient-specific responses to therapies, bridging the gap between preclinical studies and clinical outcomes. Such platforms are critical for developing precision oncology strategies, minimizing systemic toxicity, and overcoming drug resistance mechanisms.

2.3 Molecular and Genetic Mechanisms

Cell cultures provide a simplified yet powerful system to explore the molecular and genetic underpinnings of carcinogenesis. Techniques like CRISPR-Cas9 gene editing enable precise manipulation of oncogenes (e.g., *MYC*, *RAS*) and tumor suppressor genes (e.g., *TP53*, *PTEN*) to study their roles in tumor progression. Transcriptomic and proteomic analyses of cultured cells reveal dysregulated signaling pathways, such as PI3K/AKT/mTOR or Wnt/β-catenin, offering targets for therapeutic intervention. Furthermore, cancer cell lines engineered to express fluorescent reporters or biosensors allow real-time tracking of cellular processes like DNA repair or epithelial-mesenchymal transition (EMT). These models also facilitate the study of epigenetic modifications, tumor heterogeneity, and the evolution of resistance mutations, providing a deeper understanding of cancer's adaptive mechanisms.

3. Future Perspectives

The rapid evolution of cell culture technologies is reshaping the landscape of cancer research and therapy. As traditional models reach their limits, innovative approaches are emerging to address the complexity of tumor biology, drug resistance, and personalized treatment strategies. By integrating advances in bioengineering, computational biology, and regenerative medicine, researchers are poised to overcome longstanding challenges in cancer modeling and therapeutic development. This section explores groundbreaking trends, including 3D cultures, organoids, and tissue engineering, that promise to bridge the gap between laboratory discoveries and clinical applications, ultimately improving patient outcomes.

3.1 3D Cultures and Organoids

Three-dimensional (3D) cell culture systems and organoids represent a transformative leap in mimicking the *in vivo* tumor microenvironment. Unlike conventional 2D monolayers, 3D models such as spheroids, organotypic cultures, and patient-derived organoids preserve the spatial architecture, cellular heterogeneity, and biochemical gradients of native tumors. These systems are particularly valuable for studying cancers with complex stromal interactions, such as pancreatic or brain tumors, where drug penetration and hypoxia play critical roles. Microfluidic "tumor-on-a-chip" platforms further enhance these models by simulating dynamic factors like blood flow and mechanical stress, enabling real-time analysis of metastasis and therapy responses. However, challenges remain in standardizing protocols, scaling production, and ensuring reproducibility across laboratories. The integration of organoids with artificial intelligence (AI) for high-content imaging and multi-omics profiling (e.g., genomics, metabolomics) will likely accelerate drug discovery and personalized therapy selection, ushering in a new era of precision oncology.

3.2 Tissue Engineering and Regenerative Medicine

Tissue engineering is revolutionizing both cancer research and post-treatment recovery. Engineered tumor models, constructed using biocompatible scaffolds and decellularized extracellular matrices (ECMs), replicate the biomechanical and biochemical properties of human tissues, offering unprecedented insights into tumor invasion, angiogenesis, and immune evasion. Meanwhile, regenerative medicine leverages stem cell technologies—such

as induced pluripotent stem cells (iPSCs) and mesenchymal stromal cells (MSCs)—to repair tissues damaged by chemotherapy, radiation, or surgery. For instance, bioengineered skin grafts or organ-specific constructs could restore function in patients undergoing radical cancer treatments. Innovations like 3D bioprinting of vascularized tumor-immune microenvironments or "living" bioreactors for drug testing are blurring the lines between *in vitro* models and clinical reality. Ethical considerations, scalability, and long-term biocompatibility remain hurdles, but the synergy between tissue engineering, cell culture, and regenerative therapies holds immense potential to transform cancer care and improve survivors' quality of life.

Multiple-Choice Question (MCQ)

Question 1: What is the definition of cell culture?
A) The culture of whole tissues in an artificial environment.
B) The growth of isolated cells outside their natural environment.
C) The culture of microorganisms only.
D) The culture of plants in a laboratory.

Question 2: Who is considered the "father of cell culture"?
A) Claude Bernard
B) Ross Harrison
C) Alexis Carrel
D) George Gey

Question 3: What is the main characteristic of primary cells in culture?
A) They have an unlimited lifespan.
B) They are genetically modified.
C) They have a limited lifespan due to replicative senescence.
D) They are always cancerous.

Question 4 : What is the primary role of fetal bovine serum (FBS) in culture media?
A) To provide essential nutrients and growth factors.
B) To increase the viscosity of the medium.
C) To reduce the pH of the medium.
D) To prevent bacterial contamination.

Question 5: What is the main advantage of serum-free culture media?
A) They are cheaper than serum-containing media.
B) They offer better reproducibility and reduce contamination risks.
C) They are easier to prepare.
D) They do not require sterilization.

Question 6: What is the main disadvantage of using fetal bovine serum (FBS)?
A) It is difficult to obtain.
B) Its composition is undefined and varies between batches.
C) It does not contain growth factors.
D) It is toxic to cells.

Question 7: What is the role of phenol red in culture media?
A) To indicate pH changes.
B) To provide nutrients to the cells.
C) To prevent bacterial contamination.
D) To stabilize the temperature of the medium.

Question 8: What is the optimal temperature for mammalian cell culture?
A) 25°C
B) 37°C
C) 42°C
D) 30°C

Question 9 : What is the main advantage of cell lines over primary cultures?
A) They have a limited lifespan.
B) They are genetically stable.
C) They can proliferate indefinitely.
D) They are easier to isolate.

Question 10: What is the main disadvantage of primary cultures?
A) They are difficult to handle.
B) They have a limited lifespan.
C) They are always cancerous.
D) They cannot be frozen.

Question 11: What is the role of trypsin in cell culture?
A) To provide nutrients to the cells.
B) To detach adherent cells.
C) To sterilize the culture medium.
D) To indicate the pH of the medium.

Question 12: What is the main advantage of cryopreservation of cells?
A) It allows for the storage of cells for future use.
B) It increases the growth rate of cells.
C) It reduces the risk of contamination.
D) It genetically modifies the cells.

Question 13: What is the main risk of contamination in cell cultures?
A) Viruses.
B) Bacteria, yeast, and fungi.
C) Heavy metals.
D) Radiation.

Question 14: What is the role of CO_2 in cell culture incubators?
A) To maintain the pH of the culture medium.
B) To reduce the temperature of the medium.
C) To increase the viscosity of the medium.
D) To prevent contamination.

Question 15: What is the main characteristic of HeLa cells?
A) They are derived from cervical cancer.
B) They have a limited lifespan.
C) They cannot be frozen.
D) They are difficult to culture.

Question 16: What is the main advantage of defined culture media?
A) They are cheaper than serum-containing media.
B) They offer better reproducibility.
C) They do not require sterilization.
D) They are easier to prepare.

Question 17: What is the main disadvantage of defined culture media?
A) They are difficult to optimize for different cell types.
B) They are more expensive than serum-containing media.
C) They do not contain growth factors.
D) They cannot be used for cancer cells.

Question 18: What is the role of HEPES in culture media?
A) To stabilize pH.
B) To provide nutrients.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 19: What is the main advantage of cell culture over animal studies?
A) Reduced costs and ethical concerns.
B) Better reproducibility of results.
C) Ease of cell manipulation.
D) All of the above.

Question 20: What is the main disadvantage of cell culture?
A) Cells may lose their in vivo characteristics.
B) Cultures are always contaminated.
C) Cells cannot be frozen.
D) Culture media are very expensive.

Question 21: What is the role of antibiotics in culture media?
A) To prevent bacterial contamination.
B) To provide nutrients to the cells.
C) To stabilize the pH of the medium.
D) To increase the growth rate of cells.

Question 22: What is the main advantage of suspension cultures?
A) Cells adhere to the substrate.
B) Cells float freely in the medium.
C) Cultures are easier to handle.
D) Cultures are less prone to contamination.

Question 23: What is the main disadvantage of suspension cultures?
A) Cells cannot be frozen.
B) Cells adhere to the substrate.
C) Cultures are more difficult to handle.
D) Cultures are more prone to contamination.

Question 24: What is the role of DMSO in cell cryopreservation?
A) To prevent ice crystal formation.
B) To provide nutrients to the cells.
C) To stabilize the pH of the medium.
D) To increase the growth rate of cells.

Question 25: What is the main advantage of 3D cultures over 2D cultures?
A) 3D cultures better replicate the in vivo environment.
B) 3D cultures are cheaper.
C) 3D cultures are easier to handle.
D) 3D cultures are less prone to contamination.

Question 26: What is the main disadvantage of 3D cultures?
A) They are more difficult to handle.
B) They are more expensive.
C) They cannot be frozen.
D) They are more prone to contamination.

Question 27: What is the role of growth factors in culture media?
A) To promote cell proliferation.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 28: What is the main advantage of organoid cultures?
A) They replicate organ structure and function.
B) They are cheaper than 2D cultures.
C) They are easier to handle.
D) They do not require culture media.

Question 29: What is the main disadvantage of organoid cultures?
A) They are difficult to establish and maintain.
B) They are more expensive than 2D cultures.
C) They cannot be frozen.
D) They are more prone to contamination.

Question 30: What is the role of hormones in culture media?
A) To regulate cell growth and differentiation.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 31: What is the main advantage of stem cell cultures?
A) They have the potential to differentiate into various cell types.
B) They are cheaper than other cultures.
C) They are easier to handle.
D) They do not require culture media.

Question 32 : What is the main disadvantage of stem cell cultures?
A) They are difficult to differentiate.
B) They are more expensive than other cultures.
C) They cannot be frozen.
D) They are more prone to contamination.

Question 33: What is the role of attachment factors in culture media?
A) To facilitate cell adhesion to the substrate.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the growth rate of cells.

Question 34: What is the main advantage of cancer cell cultures?
A) They proliferate indefinitely.
B) They are cheaper than other cultures.
C) They are easier to handle.
D) They do not require culture media.

Question 35: What is the main disadvantage of cancer cell cultures?
A) They may lose their in vivo characteristics.
B) They are more expensive than other cultures.
C) They cannot be frozen.
D) They are more prone to contamination.

Question 36: What is the role of antioxidants in culture media?
A) To protect cells from oxidative stress.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the growth rate of cells.

Question 37: What is the main advantage of plant cell cultures?
A) They can produce bioactive compounds.
B) They are cheaper than animal cultures.
C) They are easier to handle.
D) They do not require culture media.

Question 38: What is the main disadvantage of plant cell cultures?
A) They are difficult to establish and maintain.
B) They are more expensive than animal cultures.
C) They cannot be frozen.
D) They are more prone to contamination.

Question 39: What is the role of vitamins in culture media?
A) To act as enzyme cofactors.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the growth rate of cells.

Question 40: What is the main advantage of insect cell cultures?
A) They can produce large amounts of proteins.
B) They are cheaper than animal cultures.
C) They are easier to handle.
D) They do not require culture media.

Question 41: What is the main disadvantage of insect cell cultures?
A) They are difficult to establish and maintain.
B) They are more expensive than animal cultures.
C) They cannot be frozen.
D) They are more prone to contamination.

Question 42: What is the role of amino acids in culture media?
A) To serve as building blocks for proteins.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the growth rate of cells.

Question 43: What is the main advantage of using cell culture in pharmaceutical research?
A) It allows for high-throughput drug screening.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 44: What is the main disadvantage of using cell culture in pharmaceutical research?
A) Cells may not fully replicate in vivo conditions.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 45: What is the role of glucose in culture media?
A) To provide energy for cell growth.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 46: What is the main advantage of using cell culture in vaccine production?
A) It allows for large-scale production of viruses.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 47: What is the main disadvantage of using cell culture in vaccine production?
A) It requires specialized equipment.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 48: What is the role of glutamine in culture media?
A) To serve as a precursor for DNA synthesis.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 49: What is the main advantage of using cell culture in genetic engineering?
A) It allows for the study of gene expression.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 50: What is the main disadvantage of using cell culture in genetic engineering?
A) Cells may not fully replicate in vivo conditions.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 51: What is the role of serum in culture media?
A) To provide growth factors and nutrients.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 52: What is the main advantage of using serum-free media?
A) It reduces batch-to-batch variability.
B) It is cheaper than serum-containing media.
C) It is easier to prepare.
D) It does not require sterilization.

Question 53: What is the main disadvantage of using serum-free media?
A) It is difficult to optimize for different cell types.
B) It is more expensive than serum-containing media.
C) It does not contain growth factors.
D) It cannot be used for cancer cells.

Question 54: What is the role of CO_2 in cell culture incubators?
A) To maintain the pH of the culture medium.
B) To reduce the temperature of the medium.
C) To increase the viscosity of the medium.
D) To prevent contamination.

Question 55: What is the main advantage of using cell culture in cancer research?
A) It allows for the study of cancer cell behavior.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 56: What is the main disadvantage of using cell culture in cancer research?
A) Cancer cells may lose their in vivo characteristics.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 57: What is the role of antibiotics in culture media?
A) To prevent bacterial contamination.
B) To provide nutrients to the cells.
C) To stabilize the pH of the medium.
D) To increase the growth rate of cells.

Question 58: What is the main advantage of using cell culture in virology?
A) It allows for the study of virus replication.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 59: What is the main disadvantage of using cell culture in virology?
A) Viruses may not fully replicate in vitro.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 60: What is the role of phenol red in culture media?
A) To indicate pH changes.
B) To provide nutrients to the cells.
C) To prevent bacterial contamination.
D) To stabilize the temperature of the medium.

Question 61: What is the main advantage of using cell culture in toxicology studies?
A) It allows for high-throughput screening of toxic compounds.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 62: What is the main disadvantage of using cell culture in toxicology studies?
A) Cells may not fully replicate in vivo conditions.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 63: What is the role of HEPES in culture media?
A) To stabilize pH.
B) To provide nutrients.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 64: What is the main advantage of using cell culture in regenerative medicine?
A) It allows for the production of tissues and organs.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 65: What is the main disadvantage of using cell culture in regenerative medicine?
A) It is difficult to scale up for clinical applications.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 66: What is the role of growth factors in culture media?
A) To promote cell proliferation.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 67: What is the main advantage of using cell culture in drug development?
A) It allows for high-throughput screening of drug candidates.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 68: What is the main disadvantage of using cell culture in drug development?
A) Cells may not fully replicate in vivo conditions.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 69: What is the role of insulin in culture media?
A) To regulate glucose metabolism.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 70: What is the main advantage of using cell culture in stem cell research?
A) It allows for the study of cell differentiation.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 71: What is the main disadvantage of using cell culture in stem cell research?
A) Stem cells are difficult to differentiate.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 72: What is the role of transferrin in culture media?
A) To transport iron.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 73: What is the main advantage of using cell culture in genetic engineering?
A) It allows for the study of gene expression.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 74: What is the main disadvantage of using cell culture in genetic engineering?
A) Cells may not fully replicate in vivo conditions.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 75: What is the role of fibronectin in culture media?
A) To promote cell adhesion.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 76: What is the main advantage of using cell culture in tissue engineering?
A) It allows for the creation of artificial tissues.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 77: What is the main disadvantage of using cell culture in tissue engineering?
A) It is difficult to scale up for clinical applications.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 78: What is the role of albumin in culture media?
A) To provide a surface for cell attachment.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 79: What is the main advantage of using cell culture in vaccine production?
A) It allows for large-scale production of viruses.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

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D) To increase the viscosity of the medium.

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D) All of the above.

Question 89: What is the main disadvantage of using cell culture in vaccine production?
A) It requires specialized equipment.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 90: What is the role of glutamine in culture media?
A) To serve as a precursor for DNA synthesis.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 91: What is the main advantage of using cell culture in genetic engineering?
A) It allows for the study of gene expression.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 92: What is the main disadvantage of using cell culture in genetic engineering?
A) Cells may not fully replicate in vivo conditions.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 93: What is the role of fibronectin in culture media?
A) To promote cell adhesion.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 94: What is the main advantage of using cell culture in tissue engineering?
A) It allows for the creation of artificial tissues.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 95: What is the main disadvantage of using cell culture in tissue engineering?
A) It is difficult to scale up for clinical applications.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 96: What is the role of albumin in culture media?
A) To provide a surface for cell attachment.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 97: What is the main advantage of using cell culture in vaccine production?
A) It allows for large-scale production of viruses.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Question 98: What is the main disadvantage of using cell culture in vaccine production?
A) It requires specialized equipment.
B) It is more expensive than animal testing.
C) It is more time-consuming.
D) It is less reproducible.

Question 99: What is the role of glutamine in culture media?
A) To serve as a precursor for DNA synthesis.
B) To stabilize the pH of the medium.
C) To prevent contamination.
D) To increase the viscosity of the medium.

Question 100: What is the main advantage of using cell culture in genetic engineering?
A) It allows for the study of gene expression.
B) It is cheaper than animal testing.
C) It reduces ethical concerns.
D) All of the above.

Exercises

Exercise 1: Figure 1 represents a growth curve of two cell populations.

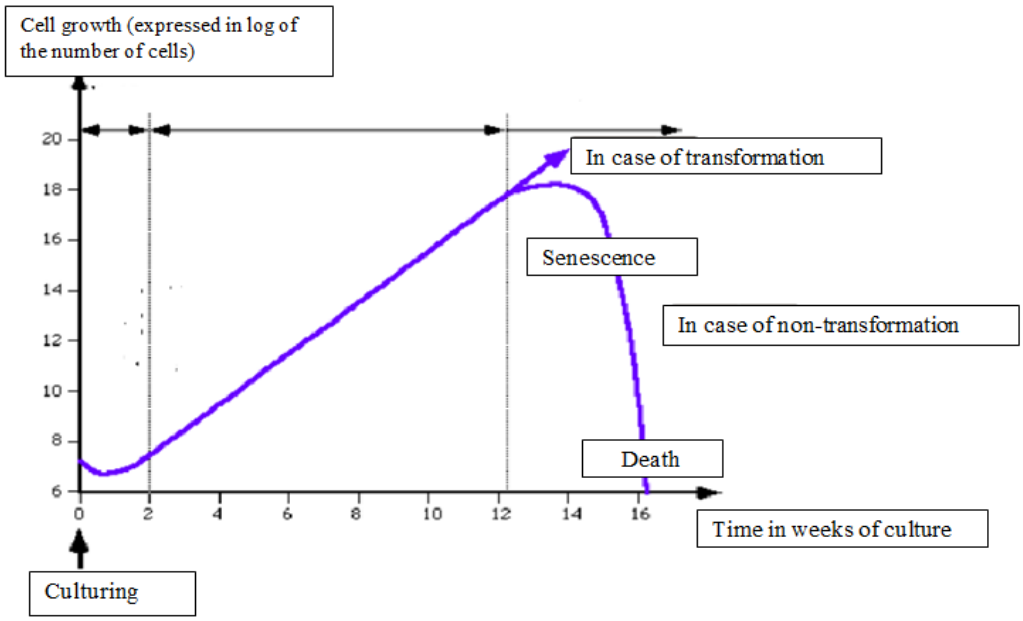
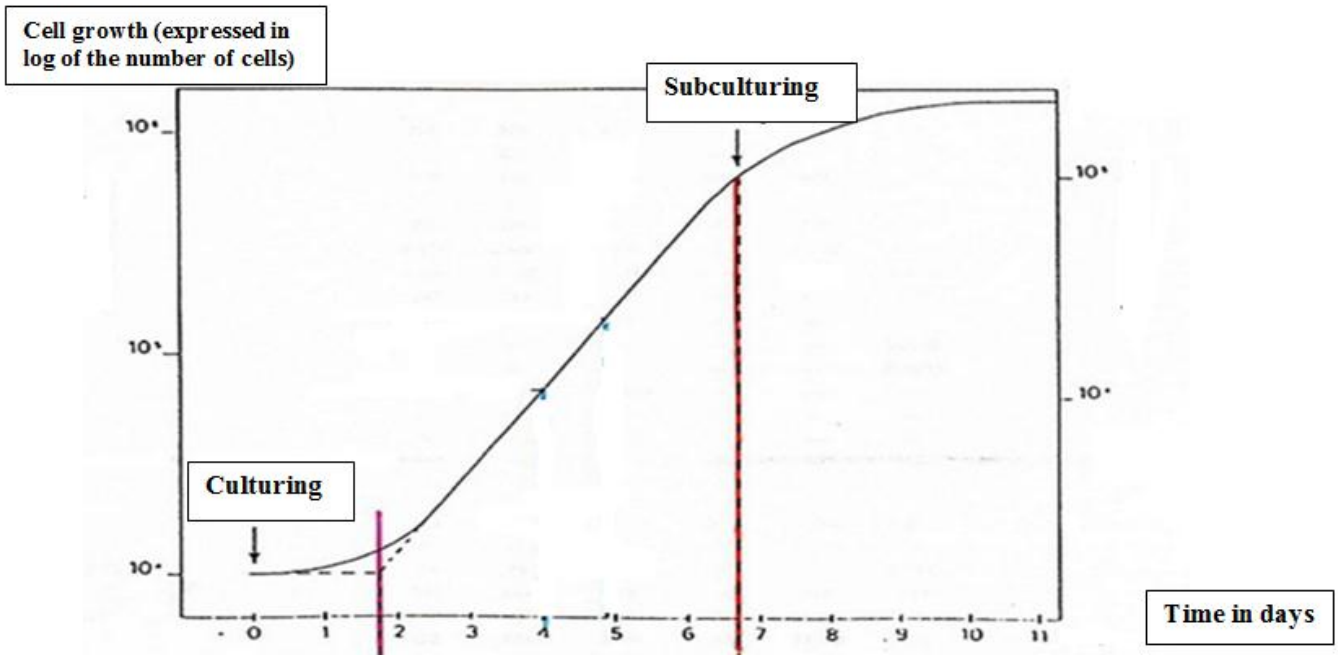


Figure 1: Evolution of a cell culture over time.

1. **Analyze and interpret the curve for non-transformed cells:**
2. **Explain the decrease to zero in the number of cells after 12 weeks.**

Exercise 2:

The diagram below represents a growth curve of a cell population (case of **transformed cells**) over time.



1. **Explain the term "transformed cells" with an example.**
2. **What are the characteristics of these transformed cells in cell culture?**
3. **Analyze and interpret the curve for transformed cells.**
4. **After the 14th day of culture, a decrease in the proliferation rate of transformed cells was observed, leading to a complete halt in cell division.**
   A. Provide an explanation for the cessation of growth
   B. What is your solution to prevent the cessation of proliferation in the cell culture of transformed cells?

Exercise 3:

Explain in a table the difference between suspension culture and adherent cell culture.

	Suspension Culture	**Adherent Cell Culture**
Definition		
Example of Cells		
Separation Method		

Solutions of MCQs :

1. B
2. B
3. C
4. A
5. B
6. B
7. A
8. B
9. C
10. B
11. B
12. A
13. B
14. A
15. A
16. B
17. A
18. A
19. D
20. A
21. A
22. B
23. C
24. A
25. A
26. A
27. A
28. A
29. A
30. A
31. A
32. A
33. A
34. A
35. A
36. A
37. A
38. A
39. A
40. A
41. A
42. A
43. D
44. A
45. A
46. D
47. A
48. A
49. D
50. A
51. A
52. A
53. A
54. A
55. D
56. A
57. A
58. D
59. A
60. A
61. D
62. A
63. A
64. D
65. A
66. A
67. D
68. A
69. A
70. D
71. A
72. A
73. D
74. A
75. A
76. D
77. A
78. A
79. D
80. A
81. A
82. D
83. A
84. A
85. D
86. A
87. A
88. D
89. A
90. A
91. D
92. A
93. A
94. D
95. A
96. A
97. D
98. A
99. A
100. D

Solution of exercice 1 :

1. **Analyze and interpret the curve for non-transformed cells:**

   This is a curve showing the evolution of the number of cells over time.

   - ✓ **0 to 1 week:** There is practically no cell growth because the cells are adapting to their new environment. This is the **adaptation phase**.

- ✓ **1 week to 12 weeks:** The cells divide rapidly as they consume most of the nutrients in the culture medium after the second subculture. This is the **rapid growth phase**.
- ✓ **12 weeks to 14 weeks:** The number of cells remains constant because as many cells die as new ones are produced. This is the **stationary phase**.
- ✓ **14 weeks to 16 weeks:** A decrease in their proliferation rate until it stops, due to the fact that cells can only multiply for a limited number of generations before they die (senescence). The cessation of cell division can also be due to the depletion of nutrients in the culture medium and a lack of space (contact inhibition). This is the **decline phase**.

2. **Explain the decrease to zero in the number of cells after 12 weeks.**

   This is due to the Hayflick limit. After 12 weeks, the cells stop dividing either because of nutrient depletion, contact inhibition (lack of space), or the Hayflick limit, as these are normal cells with a programmed cell death after approximately 50 divisions.

Solution of exercise 2:

1. **Explain the term "transformed cells" with an example.**

   Cells with an unlimited division capacity (referred to as immortality in culture) are called "cell lines." These lines can be cancer cells (such as HeLa cells, derived from Henrietta Lacks, which grow very rapidly), cells in the process of becoming cancerous, or healthy cells that have been artificially made "immortal."

2. **What are the characteristics of these transformed cells in cell culture?**

   Transformed cells in cell culture lose contact inhibition and grow in clusters or multilayers.

   - ✓ They can often be cultured in suspension (lose their dependence on a support).
   - ✓ They change morphology (become rounded).
   - ✓ Adherent cells lose their need for anchorage and can be cultured in suspension.
   - ✓ Immortality.
   - ✓ Growth autonomy.

- ✓ Independence from growth factors.

3. **Analyze and interpret the curve for transformed cells.**

   Three phases can be distinguished: the adaptation phase (Lag), the exponential phase (Log), and the stationary phase (Plateau).

   - ✓ **0 to 1.5 days: Lag phase:** Period of adaptation to the environment. The cell rebuilds its cytoskeleton, attaches to the substrate, and spreads. There is DNA and protein synthesis.
   - ✓ **1.5 to 6.5 days: Log phase:** Exponential growth phase. The cells divide rapidly as they consume most of the nutrients in the culture medium.
   - ✓ **6.5 to 11 days: Plateau phase:** The cell multiplication rate does not decrease, allowing for an indefinite number of subcultures.

   Transformed cells reach high plateaus. Having lost their dependence on a support, they can often be cultured in suspension.

4. After the 14th day of culture, a decrease in the proliferation rate of transformed cells was observed, leading to a complete halt in cell division.

A. **Provide an explanation for the cessation of growth:**

- Depletion of nutrients.

B. **What is your solution to prevent the cessation of proliferation in the cell culture of transformed cells?**

- Perform subculturing (passaging) into a fresh culture medium.

Solution of exercise 3:

The difference between suspension culture and adherent cell culture.

	Suspension Culture	**Adherent Cell Culture**
Definition	Type of culture where cells float in the medium and proliferate in suspension.	Culture on a support: cells adhere to the bottom of the flask or culture dish.
Example of Cells	Blood, bone marrow, amniotic fluid	Skin
Separation Method	Centrifugation on Ficoll	Dissection methods by grinding or enzymatic methods using proteolytic enzymes.

Glossary of Technical Terms:

1. Cell Culture Fundamentals

- **Primary Culture:**
  Initial culture established directly from tissue or organ fragments, retaining original characteristics.
- **Secondary Culture:**
  Subculture of cells derived from a primary culture, expanded for experimental use.
- **Cell Line:**
  A population of cells derived from a primary culture that can proliferate indefinitely (e.g., immortalized or cancerous cells).
- **Subculturing:**
  Transferring a portion of cells from an existing culture to fresh medium to maintain growth.
- **Cell Viability:**
  Percentage of living cells in a population, often assessed using dyes like Trypan Blue.
- **Contact Inhibition:**
  Phenomenon where adherent cells stop dividing upon forming a confluent monolayer.

2. Infrastructure & Equipment

- **Microbiological Safety Cabinet (MSC):**
  Enclosed workspace with HEPA-filtered airflow to maintain sterility during cell handling.
- **CO_2 Incubator:**
  Device maintaining 5% CO_2, 37°C, and humidity for mammalian cell growth.
- **Autoclave:**
  Equipment using high-pressure steam (121°C) to sterilize lab tools and media.
- **Laminar Flow Hood:**
  Sterile workspace with unidirectional airflow to prevent contamination.

3. Culture Media & Components

- **Fetal Bovine Serum (FBS):**
  Nutrient-rich supplement derived from fetal calf blood, providing growth factors and proteins.

- **Serum-Free Media:**
  Chemically defined culture medium without animal-derived components.
- **Phenol Red:**
  pH indicator dye in media (red at pH 7.4, yellow if acidic, purple if alkaline).
- **HEPES Buffer:**
  Chemical buffer stabilizing pH in cultures without CO_2 control.
- **Antibiotic/Antimycotic Cocktail:**
  Mixture (e.g., penicillin-streptomycin) to prevent bacterial/fungal contamination.

4. Cell Isolation & Characterization

- **Trypsinization:**
  Enzymatic dissociation of adherent cells using trypsin to detach them from surfaces.
- **Ficoll Gradient:**
  Density-based centrifugation method to isolate specific cell types (e.g., lymphocytes).
- **Cryopreservation:**
  Long-term storage of cells at ultra-low temperatures (-196°C) using cryoprotectants like DMSO.
- **Hayflick Limit:**
  Maximum number of divisions normal cells undergo before entering senescence (40–60 divisions).
- **Transfection:**
  Introduction of foreign DNA/RNA into cells to modify gene expression.

5. Contamination & Quality Control

- **Mycoplasma:**
  Bacteria-like contaminants that evade detection and disrupt cell metabolism.
- **Endotoxin:**
  Toxic molecules from bacterial membranes that induce immune responses in cultures.
- **Cross-Contamination:**
  Unintended mixing of cell lines, leading to misidentification (e.g., HeLa contamination).
- **Aseptic Technique:**
  Practices to prevent contamination (e.g., flame sterilization, glove use).

6. Advanced Techniques & Applications

- **3D Culture:**
  Growth of cells in scaffolds or matrices to mimic tissue architecture (e.g., spheroids).
- **Organoid:**
  Miniaturized, functional organ-like structures grown from stem cells.
- **Tissue Engineering:**
  Combining cells, scaffolds, and growth factors to regenerate damaged tissues.
- **CRISPR-Cas9:**
  Gene-editing tool used to modify cell genomes for research or therapeutic purposes.
- **Bioreactor:**
  Device for large-scale cell culture under controlled conditions (e.g., for vaccine production).

7. Cancer & Stem Cell Research

- **Tumorigenicity:**
  Ability of cells to form tumors in vivo, a key test for cancer cell lines.
- **Cancer Stem Cells (CSCs):**
  Subpopulation of tumor cells with self-renewal and metastatic potential.
- **Induced Pluripotent Stem Cells (iPSCs):**
  Adult cells reprogrammed to an embryonic-like pluripotent state.
- **Apoptosis:**
  Programmed cell death, distinct from necrosis (accidental cell death).

8. Emerging Fields

- **Organ-on-a-Chip:**
  Microfluidic device simulating human organ functions for drug testing.
- **Exosome:**
  Nanoscale vesicles secreted by cells, used in cell-to-cell communication studies.
- **Biobanking:**
  Systematic storage of cell lines and tissues for future research.
- **Synthetic Biology:**
  Engineering cells to perform novel functions (e.g., biosensors).

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