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Article on cell culture techniques

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Abstract
Cell culture techniques are essential in biological and biomedical research, allowing for the controlled growth of cells from multicellular organisms. They have transformed areas like molecular biology, genetics, pharmacology, cancer research, and regenerative medicine. The process involves isolating cells, growing them in nutrient-rich media, and maintaining sterile conditions. Various cell cultures exist, including primary cultures with limited lifespans and immortalized cell lines that proliferate indefinitely. Innovations like 3D culture systems and organoid models enhance the mimicry of in vivo environments, aiding the understanding of biological processes. Key techniques include subculturing, cryopreservation, transfection, and mycoplasma testing, with culture media composition being critical for cell health. However, challenges such as genetic drift, instability, and contamination risks persist, alongside ethical concerns leading to the exploration of alternatives like induced pluripotent stem cells (iPSCs). Cell culture remains a vital technique crucial for therapeutic advancements and personalized medicine.

Keywords: Culture media, equipments, cryopreservation, cryoinjury, freezing method, 3D cell culture

Introduction
Cell culture is a vital research technique in life sciences, bridging fields like cell biology, biochemistry, and medicine. It is increasingly used for diagnostic applications, molecular biology, and drug development, while modern techniques allow for the creation of specialized cells. Cell culture involves cultivating cells from animals or plants in a controlled environment, aiding in understanding cell physiology, disease mechanisms, and drug effects. Its applications extend to the production of therapeutic proteins, vaccines, and in food industries for contamination testing and cellular agriculture, contributing to sustainable practices like cultured meat production.

Types of cell culture
Cell culture is classified into three:

Primary cell culture
- Adherent cell culture
- Suspension cell culture

Secondary cell culture

Cell line
- Finite cell culture
- Continuous cell line

Primary cell culture involves separating cells from their parent tissue using a mechanical enzymatic process and maintaining them in a culture medium. There are two main types of cells in culture: adherent cells, which require attachment to surfaces to proliferate, and suspension cells, which do not need attachment and are derived from blood cells. When primary cultures are subcultured, they become secondary cultures; this process includes cell transfer and providing new nutrients. Cell lines can be classified as finite, with limited lifespan and slower growth, or continuous, which can grow indefinitely and exhibit rapid

division. Monolayer cultures consist of a single-layer of cells, while suspension cultures include non-adhesive cells that can be kept in suspension.

Cell culture equipment

- Laminar flow hood
- Incubator (humid co2 incubator recommend)
- Water bath
- Centrifuge
- Inverted microscope
- Refrigerator and freezer(-20C)
- Cell culture vessels
- Sterilizer(autoclave)

Laminar flow hood

Laminar flow hoods are essential in cell culture to maintain aseptic conditions and prevent microbial contamination. They utilize HEPA filters to remove 99.97% of particles ≥0.3 µm, ensuring a sterile workspace through unidirectional air flow. There are two main types: horizontal laminar flow hoods, which protect samples but not the operator, and vertical laminar flow hoods, which provide better protection by directing airflow downwards. These hoods are critical for media preparation, cell passaging, and other sterile techniques, significantly reducing contamination risks.

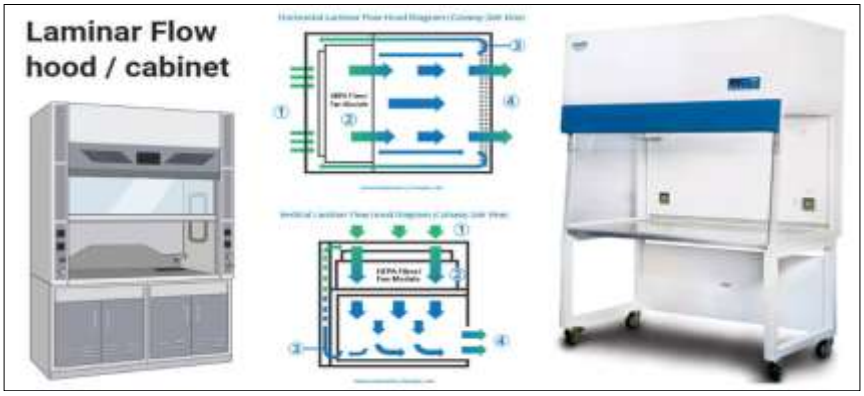

Fig 1: laminar flow hood Incubator: (humid co2 incubator recommend)

In cell culture, incubators are vital for providing controlled environments for optimal cell survival and growth. Key functions include temperature control (maintaining around 37 °C), regulation of CO_2 (typically 5-10% for pH stabilization), and high humidity (95-98%) to prevent medium evaporation. Types of incubators include water-jacketed (stable thermal conditions), air-jacketed (rapid recovery), and tri-gas incubators (control CO_2, O_2, and N_2 for specialized studies). Applications range from maintaining mammalian cell lines to drug discovery and cancer biology studies. Good practices involve minimizing door openings, regular cleaning, UV sterilization, and accurate CO_2 monitoring.

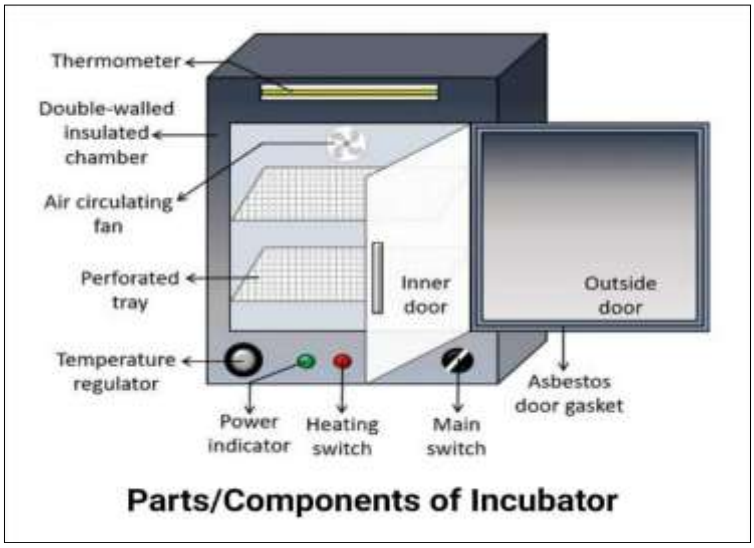

Fig 2: Incubator Inverted microscope

Invented in 1850 by J. Lawrence Smith, the inverted microscope is a type of light microscope with an atypical configuration where the light source and condenser lens are positioned above the specimen stage, while the objectives are located below, pointing upwards. This arrangement allows for viewing images from below rather than above. While it functions on the same principle as a standard upright microscope, the inverted microscope's design is particularly suitable for observing specimens placed on a large stage. The condenser lens concentrates light onto the specimen, facilitating magnification through the objectives and viewing via the ocular lens, enhanced by specialized coverslips for optimal optical performance.

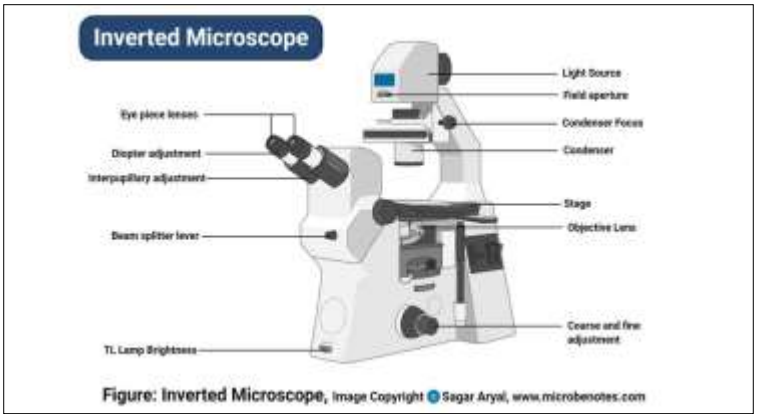

Figure 3: Inverted microscope

Centrifuge

A centrifuge is a device that utilizes centrifugal force to separate components in a fluid by spinning it at high speeds. This process causes denser substances to move outward while lighter substances rise to the center. Laboratory centrifuges settle denser particles at the bottom of sample tubes, acting as effective filters. Industrial centrifuges are employed in manufacturing and waste processing to separate suspended solids and immiscible liquids, such as in cream separators in dairies. High-speed centrifuges and ultracentrifuges can separate fine particles and molecules, while larger centrifuges simulate high gravity environments. Medium-sized centrifuges are found in washing machines and swimming pools, and gas centrifuges are used for isotope separation, particularly in nuclear fuel enrichment.

Fig 4: Centrifuge Culture media

- Culture media are nutrient pre
  parations that support the growth of microorganisms in laboratories.
- Different microbes have different nutritional needs, so no single medium is suitable for all.
- Some microorganisms (obligate parasites) cannot grow in any artificial medium.
- **Importance:** Used in diagnosis of infectious diseases, pure culture isolation, vaccine development, genetic studies, biochemical tests, contamination checks, viable counts, and antibiotic sensitivity tests.
- Main focus: Composition, classification, and types of culture media.

Classification of Culture Media

A. Based on Consistency

1. Solid media (1.5–2% agar):

a) Used to separate colonies, observe morphology, and create pure cultures.

b) Colony forms: smooth, rough, mucoid, filamentous, punctiform, etc.

c) Examples: Nutrient agar, Blood agar, MacConkey agar, Chocolate agar.

2. Semisolid media (0.2–0.5% agar)

a) Jelly-like, softer consistency.

b) Used for motility tests and growth of microaerophiles.

c) **Examples:** Hugh-Leifson's OF medium, Stuart's medium, Mannitol motility medium.

3. Liquid media (broths):

a) No solidifying agents; uniform turbidity on growth.

b) Used for bulk culture, fermentation studies.

c) **Examples:** Nutrient broth, MR-VP broth, Tryptic soy broth.

4. Biphasic media: Combination of liquid + solid.

Example: Lowenstein-Jensen medium (egg-based), Loeffler's serum slope.

B. Based on Nutritional Component

1. **Simple media:** For non-fastidious microbes.
   Examples: Nutrient agar, Peptone water.
2. **Complex media:** Contain unknown nutrient composition.
   Examples: Blood agar, Tryptic soy broth.
3. **Synthetic (defined) media:** Exact chemical composition known.
   Example: Czapek Dox medium.

C. Based on Application / Chemical Composition

1. **Basal media:** Basic carbon & nitrogen sources, non-selective.
   Examples: Nutrient broth, Nutrient agar, Peptone water.

2. **Enriched media:** Basal media + supplements (blood, serum, egg yolk).
   a) For fastidious organisms.
   b) **Examples:** Blood agar (hemolysis detection), Chocolate agar (N. gonorrhoeae), Loeffler's serum slope.

3. **Selective media:** Contain chemicals that inhibit some microbes but allow others**.**
   a) **Selective agents:** bile salts, dyes, antibiotics, pH adjustments.
   b) **Examples:** MacConkey agar (Gram-negative enterics), Mannitol salt agar (Staphylococci).

Basic components of culture media

1. Carbon source (e.g., glucose).
2. Buffering system (e.g., HEPES).
3. pH Indicator (e.g., phenol red).
4. Serum.
5. Metabolites, vitamins, and minerals.
6. Antibiotics.

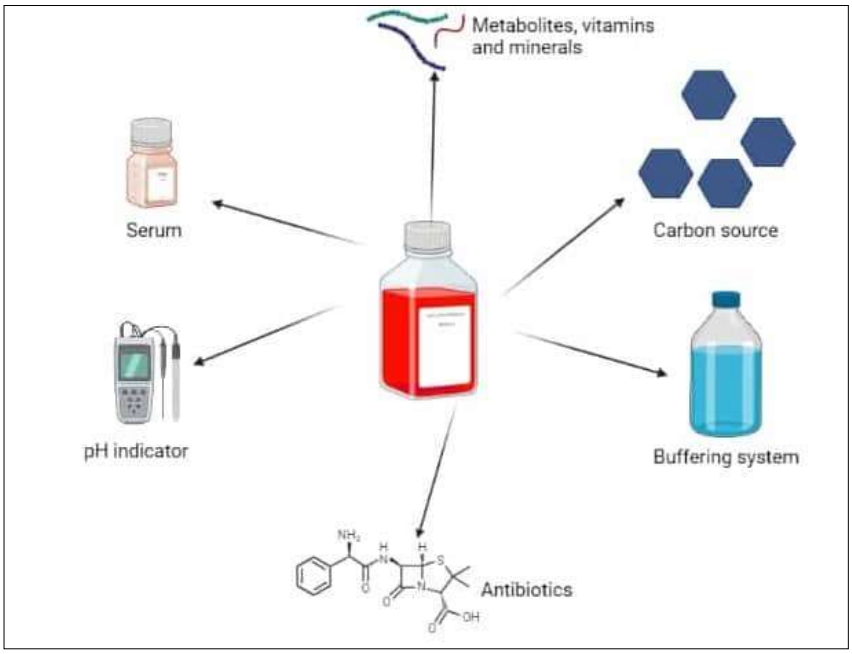

Fig 5: Composition of mammalian cell culture media

1. Carbon Source

Carbon sources are essential energy sources for cell metabolism in growth media, commonly provided by glucose at a concentration of 5.5 mM, which mirrors human blood levels. Alternatives include other sugars like galactose and fructose, as well as compounds like pyruvate and glutamine. Media can be purchased with various glucose concentrations, including glucose-free options to investigate specific physiological conditions such as high-sugar diabetes or low-glucose stress responses.

2. Buffering System

Cells require a specific pH range of 7.2 to 7.4 for optimal functioning. High CO2 levels can acidify media by producing hydrogen ions, thus necessitating a buffering system to maintain pH. Sodium bicarbonate is a common buffer that reacts with hydrogen ions, but it is not optimal at physiological pH. Researchers often add alternatives like HEPES to enhance buffering capacity in culture media.

Cell Cryopreservation

The process of using extremely low temperatures to prolong the life of structurally intact living cells and tissues is known as cryopreservation. The cryobiological reaction and cryosurvival during the freezing and thawing cycle vary greatly throughout mammalian species, depending on the cell types or specific cells. Slow freezing, vitrification—which solidifies the aqueous milieu of the cell or tissue into a noncrystalline glassy phase—subzero nonfreezing storage, and dry preservation are the broad categories into which cryopreservation procedures can be divided. Because it is difficult to introduce the glucose disaccharide (342 Da) and amino acids (used as plant preservatives), it is often not practicable to store mammalian cells in the dry condition.

Cryoinjury

The exact mechanism of cryoinjury, which damages cells due to phase changes of water in both extra- and

intracellular environments at low temperatures, remains unclear. The velocities of cooling and thawing significantly affect physicochemical and biophysical reactions, impacting survival rates. Notably, cryoinjury mechanisms, such as osmotic rupture from concentrated solutes and intracellular ice formation, are suggested to depend on the cooling rate.

CPAs

The CPA minimizes freezing injury during cryopreservation and must be biologically acceptable, cell-penetrating, and low in toxicity. Various CPAs are designed to reduce ice formation depending on cell type, cooling and warming rates. Optimizing sample volume, cooling and warming rates, and CPA concentrations is crucial for cellular and tissue survival. Additionally, the macroscopic dimensions of the tissue are critical in defining a cryopreservation protocol due to heat and mass transfer limitations.

Freezing method: conventional slow freezing and verification

Cryopreservation is achieved through slow freezing or vitrification, differing mainly in CPA concentrations and cooling rates. Slow freezing relies on a controlled cooling rate (approximately 1 °C/min) and the use of sub-1.0M CPA solutions to replace intracellular water and reduce cell damage, adapting to specific cell membrane permeability. Optimal cooling rates vary by cell type due to differences in water movement across membranes. A high-cost controlled-rate freezer or a portable benchtop freezing container is typically employed in this process.

a) Cryopreservation of cells or organs, Cryosurgery
b) Biochemistry and molecular biology
c) Food sciences, Ecology and plant physiology
d) Many medical applications, such as blood transfusion, bone marrow transplantation, artificial insemination ,and in vitro fertilization.

Applications of cryopreservation

a) Cryopreservation of cells or organs
b) Cryosurgery
c) Biochemistry and molecular biology
d) Food sciences
e) Ecology and plant physiology
f) Many medical applications, such as blood transfusion , bone marrow transplantation , artificial insemination ,and in vitro fertilization.

Advanced Cell Culture Techniques

a) 3D Cell Culture
b) Co-Culture Techniques
c) Cell Culture Automation

References

1. Pollard TD, Earnshaw WC. *Cell biology*. 2nd ed. Philadelphia: Saunders/Elsevier; 2007.
2. World Health Organization. *Laboratory biosafety manual*. 3rd ed. Geneva: WHO; 2004.
3. American Biological Safety Association. Biosafety cabinet and laminar flow hood guidelines. ABSA; 2021.
4. Thermo Fisher Scientific. CO_2 incubators: best practices for cell culture [White paper]. Thermo Fisher Scientific; 2020.
5. Pegg DE. Principles of cryopreservation. *Methods Mol Biol*. 2007;368:39-57. doi:10.1007/978-1-59745-362-2_3
6. Gao D, Critser JK. Mechanisms of cryoinjury in living cells. *ILAR J*. 2000;41(4):187-196. doi:10.1093/ilar.41.4.187
7. Mazur P, Leibo SP, Chu EH. A two-factor hypothesis of freezing injury. Evidence from Chinese hamster tissue-culture cells. *Exp Cell Res*. 1972;71(2):345-355. doi:10.1016/0014-4827(72)90303-5