Cell Culture Techniques

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- Cell culture is the process of growing and maintaining cells outside the living organism under controlled laboratory conditions.
- Cells are cultured in specially designed containers that provide suitable nutrients, temperature, pH, and environmental conditions for growth.
- Cell culture techniques are widely used in biotechnology, pharmaceutical research, toxicology, vaccine production, and drug discovery.
- Cell culture provides a controlled environment for studying the structure, function, growth, and behavior of cells.

Objectives of Cell Culture

- To study normal and abnormal cellular functions.
- To evaluate the efficacy and safety of drugs.
- To produce vaccines, therapeutic proteins, and monoclonal antibodies.
- To investigate disease mechanisms.
- To support tissue engineering and regenerative medicine.

Requirements for Cell Culture

- A sterile environment to prevent contamination.
- Suitable culture media containing essential nutrients.
- Appropriate temperature, humidity, and gas composition.
- Proper equipment and trained personnel.

Advantages of Cell Culture

- Cell culture reduces the need for animal experiments.
- Cell culture allows precise control of experimental conditions.
- Cell culture provides reproducible results.
- Cell culture supports high-throughput screening of drug candidates.

Limitations of Cell Culture

- Cell cultures are highly susceptible to microbial contamination.
- Cultured cells may not completely represent the complexity of living organisms.
- Cell culture procedures require specialized equipment and expertise.

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Basic Equipment Used in a Cell Culture Laboratory

- Cell culture laboratories require specialized equipment to maintain sterile conditions and support cell growth.
- Proper use and maintenance of equipment are essential for successful cell culture experiments.

Essential Equipment

1. Laminar Air Flow Cabinet or Biosafety Cabinet

- This equipment provides a sterile working environment by filtering air through high-efficiency particulate air (HEPA) filters.
- It protects both the cells and the laboratory personnel from contamination.

2. Carbon Dioxide (CO_2) Incubator

- The incubator maintains a controlled environment with appropriate temperature, humidity, and carbon dioxide concentration.
- Most mammalian cells are cultured at 37°C with 5% carbon dioxide.

3. Inverted Microscope

- The inverted microscope is used to observe cell growth, morphology, and contamination.
- It allows examination of cells growing at the bottom of culture vessels.

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4. Centrifuge

- The centrifuge separates cells from culture media by applying centrifugal force.
- It is commonly used during cell harvesting and washing procedures.

5. Water Bath

- The water bath maintains a constant temperature for warming media, reagents, and thawing frozen cells.

6. Refrigerators and Freezers

- Refrigerators and freezers are used for storing media, reagents, and biological samples.

7. Liquid Nitrogen Storage System

- This system is used for long-term preservation of cells at extremely low temperatures.

8. Autoclave

- The autoclave sterilizes culture media, glassware, and laboratory equipment using steam under pressure.

9. Cell Counters

- Cell counters are used to determine cell number and viability.

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- Cell counting may be performed manually or using automated instruments.

Cell Culture Media

- Cell culture media are nutrient solutions that support the growth, survival, and multiplication of cells in vitro.
- The composition of the culture medium depends on the type of cells being cultured.

Functions of Cell Culture Media

- To provide essential nutrients for cell growth.
- To maintain suitable pH and osmotic balance.
- To supply energy sources for cellular activities.
- To support cell metabolism and proliferation.

Components of Cell Culture Media

1. Inorganic Salts

- Inorganic salts maintain osmotic pressure and provide essential ions.

2. Carbohydrates

- Glucose is the primary energy source for cultured cells.

3. Amino Acids

- Amino acids are required for protein synthesis and cellular growth.

4. Vitamins

- Vitamins act as cofactors in metabolic reactions.

5. Buffering Agents

- Buffering agents maintain the pH of the culture medium.
- Sodium bicarbonate and HEPES are commonly used buffers.

6. Serum

- Serum provides growth factors, hormones, proteins, and attachment factors.
- Fetal bovine serum is commonly used in cell culture.

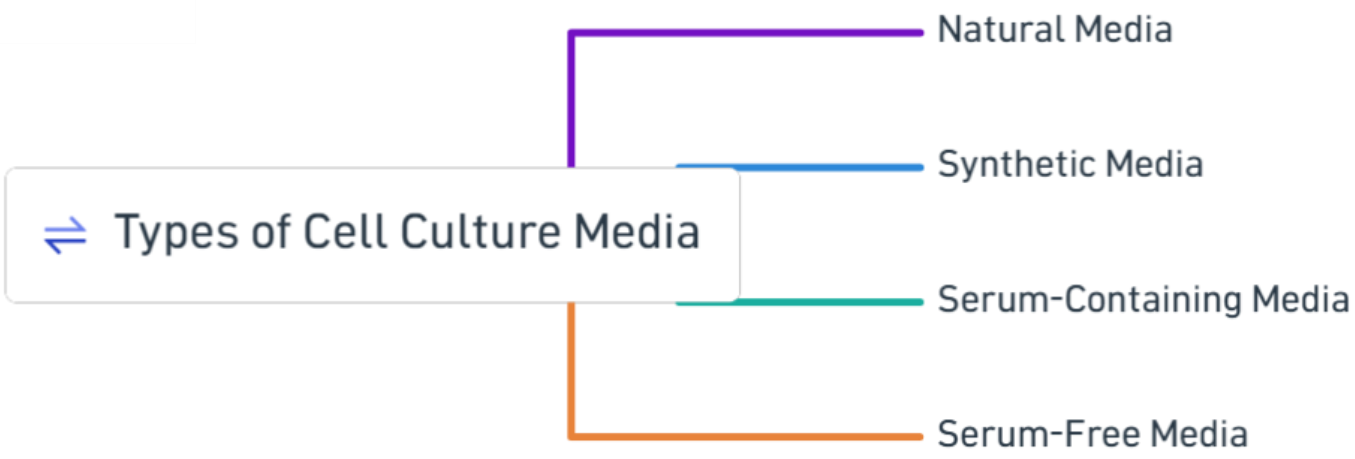
7. Antibiotics

- Antibiotics help prevent bacterial contamination.
- Excessive use of antibiotics should be avoided because they may mask poor aseptic techniques.

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Types of Cell Culture Media



A. Natural Media

- Natural media are derived from biological sources such as serum, plasma, and tissue extracts.

B. Synthetic Media

- Synthetic media are prepared using defined chemical components.

C. Serum-Containing Media

- These media contain serum to support cell growth.

D. Serum-Free Media

- These media contain defined components instead of serum.
- Serum-free media improve reproducibility and reduce variability.

Commonly Used Media

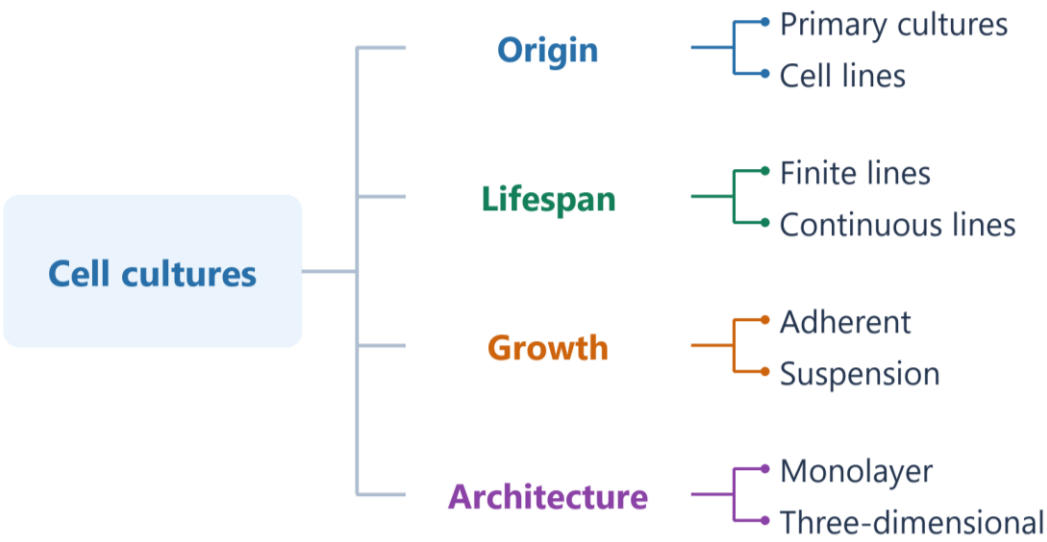
- Dulbecco's Modified Eagle Medium (DMEM).
- Roswell Park Memorial Institute Medium (RPMI-1640).
- Minimum Essential Medium (MEM).
- Ham's F-12 Medium.

Various Types of Cell Culture

- Cell cultures are classified according to the origin, growth characteristics, and lifespan of the cells.
- Different types of cell cultures are selected based on research objectives.

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Ways to classify cell cultures



Cell cultures can be described using several independent classification features

Classification Based on Origin

1. Primary Cell Culture

- Primary cultures are prepared directly from tissues or organs.
- These cells closely resemble their original tissue characteristics.
- Primary cells have a limited lifespan.

2. Cell Lines

- Cell lines are obtained from primary cultures after repeated subculturing.
- Cell lines can be maintained for extended periods under suitable conditions.

Classification Based on Lifespan

A. Finite Cell Lines

- Finite cell lines undergo a limited number of cell divisions before they stop growing.

B. Continuous Cell Lines

- Continuous cell lines can divide indefinitely under appropriate conditions.
- These cells are often derived from tumors or transformed cells.

Classification Based on Growth Characteristics

1. Adherent Cell Cultures

- Adherent cells require attachment to a solid surface for growth.
- Most cells derived from tissues grow as adherent cultures.

2. Suspension Cell Cultures

- Suspension cells grow freely in liquid media without attachment.

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