Chapter 8

Animal Cell Culture

EXERCISES

1. What is animal cell culture?

Ans: Animal cell culture is the technique of growing animal cells in a controlled, artificial environment outside of an organism. This involves providing them with nutrients, growth factors, and the right temperature and atmosphere to thrive. It's a powerful tool used in various fields like research, vaccine production, and even meat cultivation.

2. Describe animal cell culture media and their types.

Ans: Animal Cell Culture Media: A Recipe for Cell Survival

Animal cell culture media are complex mixtures designed to mimic the natural environment of animal cells and provide them with all the essential nutrients and signals they need to survive and thrive outside of an organism. These "liquid life support systems" play a crucial role in various applications, from basic research to vaccine production and even tissue engineering.

Components of Animal Cell Culture Media:

***Basal Medium:** This forms the backbone, providing essential salts, vitamins, amino acids, carbohydrates (like glucose), and energy sources (like glutamine). Examples include Dulbecco's Modified Eagle Medium (DMEM) and Ham's F-12 Nutrient Mixture.

***Supplements:** These additions cater to specific cell requirements and optimize growth. They can include:

.Serum: Bovine or fetal bovine serum (FBS) is a common source of growth factors, hormones, and attachment factors, but serum-free alternatives are gaining popularity due to ethical and batch-to-batch variation concerns.

.Growth factors: Specific proteins like insulin, epidermal growth factor (EGF), and fibroblast growth factor (FGF) stimulate cell proliferation and differentiation.

.Antibiotics and antifungals: Prevent contamination by bacteria and fungi.

.Buffers: Maintain a stable pH balance.

Types of Animal Cell Culture Media:

***Serum-containing media:** Traditional workhorses, but face concerns about batch-to-batch variation, potential contaminants, and ethical considerations.

***Serum-free media:** A growing trend, reducing ethical and contamination concerns, but require careful optimization for specific cell types.
***Defined media:** Precisely defined formulations with known components, ideal for studying specific metabolic pathways or designing synthetic biomolecules.
***Conditioned media:** Used media from healthy cultures can be supplemented to support growth of new cultures, particularly for demanding cell types.
Choosing the Right Media:
The ideal medium depends on several factors, including:
***Cell type:** Different cell lines have specific nutrient requirements and growth preferences.
***Culture conditions:** Factors like oxygen tension, pH, and temperature need to be considered.
***Application:** Research studies may require a different media composition than cell line production.
Conclusion:

Animal cell culture media are intricate and adaptable recipes, constantly evolving to meet the diverse needs of different cell types and applications. Understanding their components and how to choose the right one is crucial for successful cell culture experiments and unlocking the potential of this powerful technology.

3. Write the advantages and disadvantages of serum in the culture media.

Ans: Advantages of Serum in Culture Media:
***Rich source of growth factors:** Serum contains a natural cocktail of hormones, growth factors, and attachment factors that stimulate cell proliferation, differentiation, and viability. This makes it particularly effective for supporting fastidious or demanding cell lines.
***Reduced need for complex additives: T**he diverse components of serum can often compensate for the need to add specific growth factors or supplement the media with numerous individual ingredients. This simplifies media preparation and reduces costs.

***Enhanced cell signaling:** Serum provides natural signaling molecules that mimic the in vivo environment, promoting optimal cell behavior and function in many cases.

***Improved cell attachment and morphology:** Serum components can facilitate cell adhesion to culture surfaces and promote normal cell morphology and organization.

Disadvantages of Serum in Culture Media:

***Variability and lack of consistency:** Serum is a natural product, and its composition can vary significantly between batches and animal sources. This inconsistency can impact cell growth and reproducibility of experiments.

***Ethical concerns:** Serum production involves using blood from animals, often fetal bovine serum (FBS), raising ethical concerns and animal welfare issues.

***Potential contamination:** Serum can harbor contaminants like viruses, bacteria, and mycoplasma, posing a risk to cell cultures and downstream applications.

***Undefined components:** The complex mixture of molecules in serum makes it difficult to identify and optimize specific components for targeted cell support, limiting control over the culture environment.

***Costly:** High-quality FBS can be expensive, particularly for large-scale cultures or long-term experiments.

Conclusion:

Serum remains a widely used component in animal cell culture media due to its effectiveness and ease of use. However, its disadvantages, particularly variability and ethical concerns, have driven the development of serum-free media alternatives. The choice between serum and serum-free media ultimately depends on the specific needs of the cell line, the application, and the ethical considerations involved in the research.

4. Describe any two chemically synthesised media.

Ans: Sure, here are two examples of chemically synthesized media:

1. DMEM (Dulbecco's Modified Eagle Medium):

*Developed in the 1950s as a modification of Basal Medium Eagle (BME), specifically for culturing chick embryo fibroblasts.

*Widely used for a variety of mammalian cell lines, including fibroblasts, epithelial cells, and cancer cells.

*Contains essential salts, vitamins, amino acids, carbohydrates (like glucose), and energy sources (like glutamine).
*Supplemented with serum, growth factors, antibiotics, and antifungals for optimal cell growth.
*Considered a "basal medium" because it can be further modified to meet the specific needs of different cell types.

2. CDMEM (Chemically Defined MEM):

*A serum-free version of MEM (Minimum Essential Medium).
*Contains precisely defined components with known chemical structures and concentrations.
*Eliminates the variability and ethical concerns associated with serum.
*Ideal for studying specific metabolic pathways or designing synthetic biomolecules.
*May require additional optimization for specific cell types due to the absence of growth factors and other complex components found in serum.

These are just two examples of the many chemically synthesized media available for animal cell culture. The choice of medium depends on the specific needs of the cells being cultured and the application.

Here are some additional points to consider:

*Chemically synthesized media are generally more expensive than serum-containing media.
*They may require more optimization for specific cell types.
*However, they offer greater consistency and control over the culture environment, and they avoid the ethical concerns associated with serum.

5. What is primary cell culture? Also discuss as to how it is developed.

Ans: Primary Cell Culture: Cells From the Source

Primary cell culture involves the isolation and growth of cells directly from living organisms, as opposed to using established cell lines. These "fresh" cells offer unique advantages and challenges compared to their immortalized counterparts, making them valuable tools in various research and biotechnological applications.

Developing a Primary Cell Culture:

1. Tissue Harvest: The initial step involves obtaining the desired tissue from an organism. This can be anything from a small biopsy to whole organs, depending on the type of cells needed. Sterile conditions are crucial to prevent contamination.
2. Disaggregation: The harvested tissue needs to be broken down into smaller pieces or individual cells. This can be achieved through mechanical (chopping, scraping), enzymatic (digestive enzymes), or a combination of methods.
3. Purification and Isolation: Depending on the desired cell type, additional steps like filtration, centrifugation, or density gradient centrifugation may be required to isolate the target cells from unwanted tissue debris and other cell types.
4. Culture Conditions: The isolated cells are then placed in a nutrient medium specifically designed for their needs. This includes providing essential salts, sugars, amino acids, and growth factors to support their survival and proliferation. Additional factors like temperature, pH, and oxygen levels are also optimized for the specific cell type.
5. Maintenance and Passage: Primary cells have a limited lifespan and undergo senescence, ultimately dying in culture. To maintain a thriving culture, cells need to be regularly split (passaged) and transferred to fresh media before they reach confluence (cover the entire culture surface).
Advantages of Primary Cell Cultures:
***Authenticity:** They retain the physiological and biochemical characteristics of the original tissue, making them more representative of in vivo conditions than established cell lines.
***Functional studies:** They can be used to study specific cell types and their unique functions, providing valuable insights into organ development, disease processes, and drug responses.
***Drug discovery:** They are essential for testing the efficacy and safety of new drugs in a more relevant model compared to immortalized cell lines.
***Tissue engineering:** Primary cells can be used to generate complex tissues for regenerative medicine applications and transplantation.
Challenges of Primary Cell Cultures:
***Heterogeneity:** Primary cell cultures are often heterogeneous, containing a mixture of different cell types from the original tissue. This can complicate data interpretation and require specific isolation techniques.

***Limited lifespan:** Unlike immortalized cell lines, primary cells have a finite lifespan and cannot be propagated indefinitely. This requires careful planning and timely experiments.
***Variability:** Primary cell cultures can vary significantly between batches due to individual differences in the donor organism and the isolation process. This requires proper controls and replicates in experiments.

Overall, primary cell culture is a powerful tool with unique advantages and challenges. Understanding its development and limitations is crucial for utilizing its potential in various research fields and biotechnological applications.

6. What is subculture or passaging of cell?

Ans: Subculture, also known as passaging, is a crucial process in cell culture that involves transferring a portion of growing cells from a culture vessel to a fresh one with new nutrient medium. Essentially, it's like giving your cell community a new home and fresh groceries so they can continue to grow and thrive.

Here's a breakdown of the key aspects of subculture:

Why Do We Subculture?

***Maintain cell growth:** Cells have a limited lifespan in culture, eventually reaching a state called confluence, where they cover the entire surface of the culture vessel. Subculture provides them with more space and fresh nutrients to continue dividing and growing.
***Dilute waste products:** As cells grow and metabolize, they produce waste products that can accumulate in the media, eventually becoming toxic. Subculture dilutes these waste products and provides fresh resources for optimal cell health.
***Generate more cells:** Subculture allows you to expand your cell population, which is useful for large-scale experiments, sharing with other researchers, or generating cell lines for specific applications.
***Maintain heterogeneity:** In some cases, subculture can help maintain the diversity of cell types within a culture, preventing the overgrowth of one type over another.

How Do We Subculture?

The specific steps of subculture vary depending on the cell type and culture conditions, but generally involve the following:

1. Prepare the new culture vessel: Sterilize a new flask or dish with fresh culture medium. The amount of medium should be appropriate for the expected cell density after splitting.
2. Detach the cells: Depending on the type of cell, this may involve enzymatic treatment, mechanical scraping, or simply rinsing the cells off the surface.
3. Count the cells: This helps determine the appropriate dilution factor for the new culture. Manual or automated cell counting methods can be used.
4. Seed the cells: Transfer the desired number of cells to the new culture vessel with fresh medium. The ideal cell density depends on the specific cell type and growth rate.
5. Incubate under optimal conditions: Place the culture vessel in the incubator with the appropriate temperature, CO2 level, and humidity for the specific cell type.
6. Monitor and repeat: Regularly monitor the growth of the cells and subculture again when they reach confluence or show signs of stress.
Challenges and Considerations:
***Contamination:** Maintaining sterile conditions throughout the subculture process is crucial to prevent contamination by bacteria, fungi, or other unwanted organisms.
***Cell stress:** The detachment and dilution process can be stressful for cells, potentially impacting their growth or viability. Optimizing the technique and using gentle methods is important.
***Loss of differentiation:** In some cases, repeated subculture can lead to a loss of differentiation in certain cell types. Maintaining specific factors in the medium or using special culture conditions may be necessary for differentiated cell lines.

Overall, subculture is a fundamental technique in cell culture that allows researchers to maintain and expand their cell populations for various purposes. Understanding the principles and considerations involved is key to performing successful subcultures and maximizing the potential of your cell cultures.

7. Differentiate between finite and continuous cell lines.

Ans: Finite and continuous cell lines are two distinct types of cell cultures used in research and biotechnology. Here's a breakdown of their key differences:

Lifespan:

***Finite cell lines:** These cultures can undergo only a limited number of population doublings, typically between 20 and 80, before they senesce and stop dividing.

***Continuous cell lines:** Also known as immortalized cell lines, they have acquired the ability to divide indefinitely, enabling them to be maintained in culture for prolonged periods.

Origin:

***Finite cell lines:** Derived directly from primary cultures of cells isolated from various tissues of multicellular organisms. They retain some characteristics of the original tissue but lose them over time in culture.

***Continuous cell lines:** Often arise spontaneously from finite cell lines due to mutations or genetic alterations that activate telomerase, an enzyme that prevents chromosome shortening and replicative senescence. Alternatively, they can be deliberately created through genetic manipulation techniques.

Growth rate:

***Finite cell lines:** Generally have slower growth rates compared to continuous cell lines due to their limited replicative capacity.

***Continuous cell lines:** Often exhibit faster growth rates and easier handling because of their indefinite proliferative potential.

Stability:

***Finite cell lines:** Can exhibit changes in gene expression and phenotype over time due to accumulated mutations and epigenetic modifications. This can impact their usefulness for research.

***Continuous cell lines:** Can be more genetically and phenotypically stable than finite cell lines, making them preferable for some studies requiring consistency.

Applications:

***Finite cell lines:** Useful for studying early stages of development, differentiation processes, and modeling certain diseases with limited progression.

***Continuous cell lines:** Widely used in various research fields, including drug discovery, toxicology, gene function analysis, and vaccine production, due to their prolonged availability and ease of cultivation.

Choosing the right cell line type depends on the specific research needs and considerations. Understanding the advantages and limitations of both finite and continuous cell lines is crucial for selecting the most suitable tool for your experiments.

8. How is cell viability measurement performed?

Ans: Cell viability measurement is a crucial step in various biological and medical research applications. It tells us how many cells in a population are alive and functioning, helping us assess the success of cell cultures, treatments, or experimental manipulations. There are several methods available, each with its own advantages and limitations. Here's an overview of some common methods:

Dye Exclusion Methods:

***Trypan Blue:** This blue dye is excluded by healthy cells with intact membranes, staining only dead cells with compromised membranes. Counting stained and unstained cells under a microscope allows you to calculate the percentage of viable cells. Simple and inexpensive, but can be subjective and labor-intensive.

***Fluorescent Dyes:** Dyes like propidium iodide or Calcein AM penetrate dead cells and emit fluorescence, while surviving cells remain unstained or fluoresce differently. Measured on a flow cytometer or fluorescence microscope, these methods offer fast and accurate quantification but require specialized equipment.

Metabolic Activity Indicators:

***MTT Assay:** This colorimetric assay measures the conversion of a yellow tetrazolium salt (MTT) to a purple formazan product by metabolically active cells. The amount of formazan produced reflects the number of viable cells and can be quantified using a spectrophotometer. Simple and widely used, but sensitive to culture conditions and requires incubation time.

***ATP Assay:** ATP, the primary energy currency of cells, is measured using luciferase enzymes that emit light in proportion to ATP levels. This method gives a rapid and sensitive readout of viability but requires specific reagents and equipment.

Other Methods:

***Cell Counting:** Counting cells manually or using an automated cell counter can be used for simple viability assessments. However, it doesn't distinguish between live and dead cells and doesn't provide information on metabolic activity.

***Electrical Impedance Sensing:** Measuring the electrical resistance across a cell culture can differentiate between viable and non-viable cells, offering a real-time and label-free method. However, it requires specialized equipment and interpretation of results.

Choosing the best method depends on several factors:

***Cell type:** Some methods are more suited for specific cell types than others.

***Sensitivity:** Some methods offer greater sensitivity to subtle changes in viability.

***Throughput:** If large sample numbers need to be assessed, high-throughput methods like flow cytometry may be preferred.

***Cost and equipment:** Consider the available resources and budget constraints.

Overall, cell viability measurement is a critical tool in many fields. Understanding the different methods and their characteristics allows researchers to choose the most appropriate one for their specific needs and ensure reliable assessment of their cell cultures, experiments, and applications.

9. Write a detailed account of application of cell culture.

Ans: The Marvelous World of Cell Culture: Applications From Research to Reality

Cell culture, the art of nurturing cells outside their natural environment, has become a cornerstone of modern biology and biotechnology. Its applications permeate various fields, contributing to research, medicine, and even the future of food production. Let's dive into the diverse roles cell cultures play in shaping our world:

1. Research and Development:

***Understanding fundamental biology:** Studying isolated cell lines allows researchers to dissect cellular processes, gene function, and disease mechanisms with unparalleled precision. This unlocks secrets of development, aging, and various pathologies.

***Drug discovery and testing:** Cultured cells provide platforms for screening countless drug candidates, identifying potential therapies for cancer, infectious diseases, and other ailments. This accelerates drug development and reduces reliance on animal models.

***Toxicology and safety testing:** Assessing the toxicity of chemicals, cosmetics, and environmental pollutants on cultured cells offers a faster and more ethical

alternative to animal testing. This ensures consumer safety and protects the environment.

***Personalized medicine:** Culturing patient-derived cells enables personalized disease modeling and drug sensitivity testing. This paves the way for tailored therapies and improved treatment outcomes.

2. Medical Applications:

***Vaccine production:** Many vaccines, like those for polio and rabies, are produced using cell cultures as hosts for virus replication. This safe and efficient method helps prevent infectious diseases on a global scale.

***Tissue engineering and regenerative medicine:** Cultured cells can be used to engineer artificial tissues and organs for transplantation, offering hope for patients suffering from organ failure or damaged tissues.

***Gene therapy:** Genetically modified cells can be introduced into patients to treat genetic diseases. Cell culture methods play a crucial role in developing and testing these groundbreaking therapies.

***Cancer research and treatment:** Studying cancer cells in culture helps develop new cancer therapies and personalized treatment strategies. Understanding tumor cell behavior also aids in cancer diagnosis and prognosis.

3. Beyond Medicine:

***Food production:** Cultured meat, grown from animal cells, offers a sustainable and ethical alternative to conventional meat production. Cell culture technology has the potential to revolutionize the food industry and reduce environmental impact.

***Biotechnology and biomaterials:** Cultured cells can be used to produce valuable bioproducts like enzymes, hormones, and antibodies for various industrial and medical applications.

***Environmental monitoring:** Cell cultures can be used as biosensors to detect environmental pollutants and toxins, contributing to environmental protection and sustainability efforts.

Challenges and Considerations:

While cell culture offers immense potential, it also faces challenges. Maintaining sterile conditions, optimizing culture conditions for specific cell types, and ensuring the validity of results are crucial considerations. Additionally, ethical

concerns regarding animal-derived serum and the potential risks of artificial meat production need careful consideration.

Looking Ahead:

The future of cell culture is bright. Advancements in stem cell research, organoid technology, and microfluidic devices promise even more sophisticated applications. With responsible development and ethical considerations, cell culture will continue to be a powerful tool for unlocking scientific breakthroughs and shaping a healthier, more sustainable future for all.

10. The example of animal cell culture media is:
(a) DMEM
(b) MS media
(c) LB Media
(d) All of the above
Ans: (a) DMEM.

11. Name the type of culture which is prepared by inoculating directly from the tissue of an organism to culture media.
(a) Primary cell culture
(b) Secondary cell culture
(c) Cell lines
(d) Transformed cell culture
Ans: (a) Primary cell culture.

12. Sodium bicarbonate is added to animal cell culture media to
(a) keep cells stuck to the plastic
(b) promote the uptake of CO_2 into animal cells
(c) maintain the correct pH when CO_2 is present
(d) keep iron soluble
Ans: (c) maintain the correct pH when CO_2 is present

13. Which of the following is NOT present in growth medium for animal cell culture?
(a) Inorganic salts
(b) Bicarbonate
(c) Carbon source
(d) Starch
Ans: (d) Starch.

14. Disaggregating of cells can be performed by:
(a) Physical disruption
(b) Enzymatic digestion
(c) Treating with chelating agents
(d) All of the above
Ans: (d) All of the above.

15. The approach in which genes are transferred into animals to obtain a large scale production of the proteins encoded by these genes in the milk, blood, etc., is called
(a) In situ culture
(b) Molecular pharming
(c) Gene therapy
(d) Hybridoma technology
Ans: (b) Molecular pharming.

16. Which of the following is a protein free animal cell culture media?
(a) RPMI-1640 Media
(b) MS media
(c) LB media
(d) None of the above
Ans: (d) None of the above.

17. MTT assay is used for
(a) Cell viability test
(b) Monitoring of variation in pH of culture media
(c) Transformation screening
(d) Cell dissociation from substratum
Ans: (a) Cell viability test.

18. Passaging of animal cells in animal cell culture is
(a) Sub-culturing of the cells
(b) Isolation of cells
(c) Passing the cells from culture tube to Petri dishes
(d) Counting of cells
Ans: (a) Sub-culturing of the cells.

19. Which of the following is NOT the major function of serum?
(a) Enhance cell attachment
(b) Stimulate cell growth
(c) Promotion of tuber and bulb formation
(d) Provide transport proteins
Ans: (c) Promotion of tuber and bulb formation.

20. Assertion: Serum is the most important component of culture media.
Reason: Serum is a good source of nutrients and also helps in cell proliferation and cell-matrix attachment.
(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.
(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.
(c) Assertion is true but reason is false.
(d) Both assertion and reason are false.
Ans: (b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.

21. Assertion: Cell lines derived from the primary culture of normal cells are finite cell line.
Reason: Some cells of the finite cell line undergoes transformation and retain the ability to divide indefinitely.
(a) Both assertion and reason are true and the reason is the correct explanation of the assertion.
(b) Both assertion and reason are true but the reason is not the correct explanation of the assertion.
(c) Assertion is true but reason is false.
(d) Both assertion and reason are false.
Ans: (c) Assertion is true but reason is false.

SUMMARY

• Animal cell culture is in vitro maintenance and proliferation of animal cells using an appropriate nutrient media.
• The basic requirement for optimal growth of cells include temperature, pH and appropriate growth medium.
• Animal cell media can be categorised into two major categories, namely natural media and artificial or synthetic media.
• Natural media consists of naturally occurring biological fluids, such as plasma, tissue extract, etc., and it is suitable for the culture of a wide range of animal cells.
• Artificial or synthetic media is made up of various nutrients (both organic and inorganic), vitamins, salts, O2 and CO2 gases, serum, carbohydrates, cofactors, etc., and it can be modified according to purpose and it is divided into four categories namely, serum containing, serum free, chemically defined and protein free media.
• The cell culture can be classified as primary cell culture and secondary cell culture (cell line).
• Primary cells can grow either as an adherent monolayer or in a suspension.
• Secondary cell lines are derived from primary cells after sub-culturing.
• Based on the life span of culture, the cell lines are categorised into finite and continuous cell lines.
• The cell viability measurement i.e., the determination of living or dead cells, is

very significant in cell culture and is done via dye exclusion or viability assays and metabolic viability assays.

• Cell culture technology has application in various areas, such as molecular genetics, immunological analyses, gene therapy, bioengineering, pharmaceutical industry, etc.

• Animal cell culture plays an important role in research and development of drug and also helps to improve the health and quality of life of patients suffering from various diseases, such as cancer, genetic disorders, etc.