SECOND UNIT- ANIMAL BIOTECHNOLOGY

INITIATION OF CELL CULTURE

The initiation of cell culture involves deriving cells from tissue through enzymatic or mechanical treatments. This process, known as primary culture, selectively results in a relatively uniform cell line. The selection process is based on the cells' ability to survive as monolayer cultures by adhering to substrates or as suspension cultures.

In cultured cells, some can grow and proliferate while others may not survive in the culture environment. Cells in monolayer cultures continue to grow until they occupy the available substrate. The term "confluence" is used when cultured cells make close contact with one another, fully utilizing the available growth area. Certain cells, sensitive to growth limitation due to density, stop growing once confluence is reached. However, transformed cells are insensitive to confluence and continue to overgrow.

When the culture becomes confluent, the cells exhibit the following characteristics:

1. They closely resemble the morphology of the tissue of origin (i.e., parent tissue).
2. They express specialized functions comparable to those of the native cells.

PRIMARY CELL CULTURE

Primary culture involves culturing techniques carried out after cell isolation but before the first subculture. Primary cultures are usually prepared from large tissue masses and may contain a variety of differentiated cells, such as fibroblasts, lymphocytes, macrophages, and epithelial cells.

The following criteria are important for the efficient development of primary cultures:

- **Embryonic Tissues:** Preferred over adult tissues due to easier disaggregation, higher yield of viable cells, and rapid proliferation in vitro.
- **Cell Quantity:** A higher number of cells should be used in primary culture since their survival rate is lower compared to subcultures.
- **Minimizing Damage:** Tissues should be processed with minimal damage to cells, and dead cells should be removed.
- **Medium Selection:** A nutrient-rich medium is advisable, with foetal bovine serum preferred over calf or horse serum.
- **Enzyme Removal:** Enzymes used for disaggregation should be removed by centrifugation.

TECHNIQUES FOR PRIMARY CULTURE

Three common techniques for primary culture of isolated tissues are:

1. **Mechanical Disaggregation:** Physically breaking down tissues.
2. **Enzymatic Disaggregation:** Using enzymes to break down tissues.

3. **Primary Explant Technique:** Developed by Harrison in 1907, this technique involves finely chopping tissue, washing it, spreading it over a growth surface, and incubating with appropriate medium until cell outgrowth is observed.

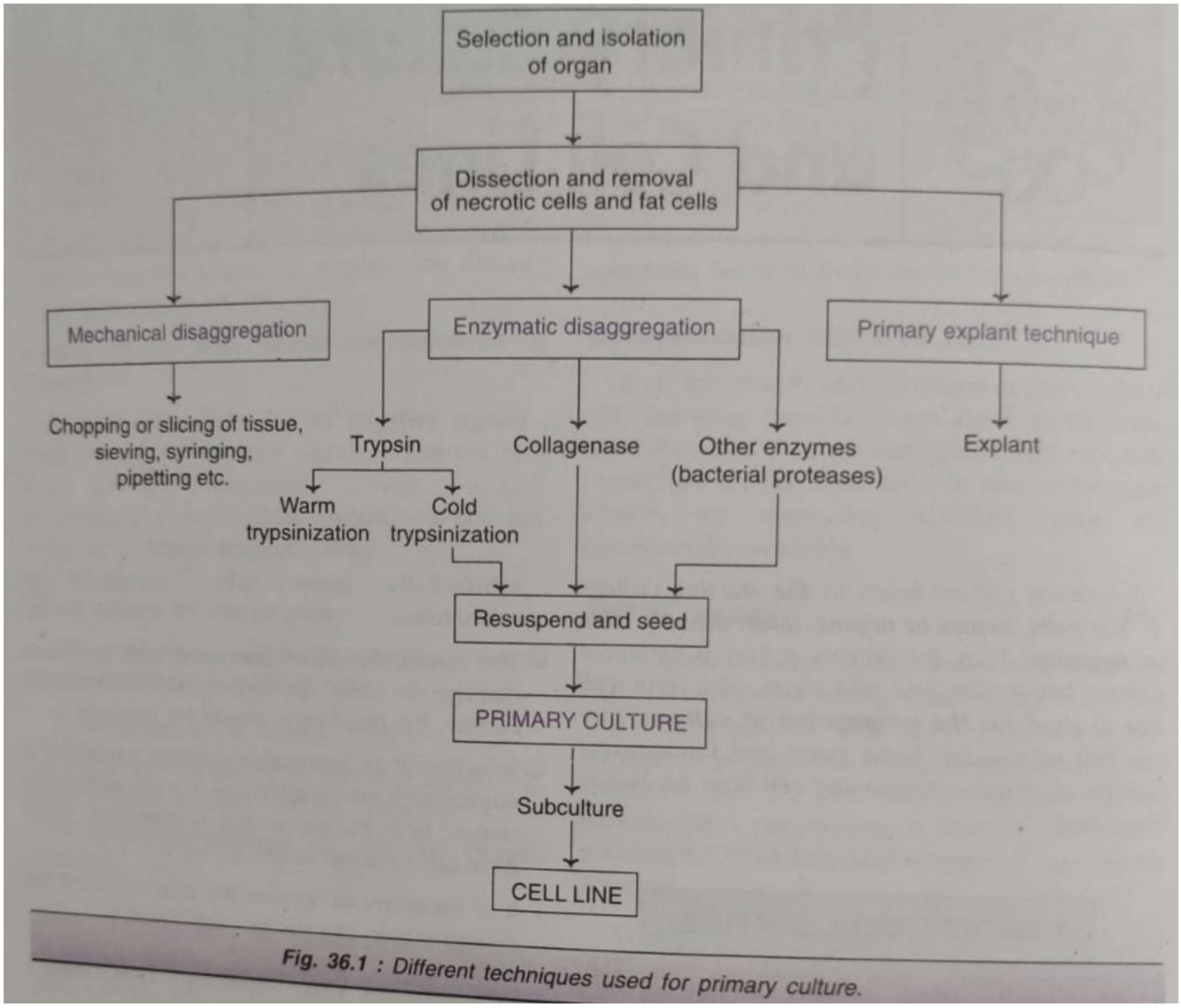

Fig. 36.1 : Different techniques used for primary culture.

PRIMARY EXPLANT TECHNIQUE

The primary explant technique, initially developed by Harrison in 1907, remains a foundational method that has evolved through various modifications and continues to be employed today. Fig. 36.4 illustrates a simplified procedure for primary explant culture, which is described briefly below.

The tissue is finely chopped in a basal salt solution and subsequently washed by settlings. Following this, the basal salt solution is removed, and the tissue pieces are evenly spread across the growth surface. After adding the appropriate medium, the culture is then incubated for 3-5 days. Subsequently, the medium is changed weekly until a significant outgrowth of cells is observed. At this stage, the explants are transferred to a fresh culture vessel.

The primary explant technique is particularly valuable for disaggregating small amounts of tissues, such as skin biopsies. Unlike mechanical or enzymatic disaggregation techniques, which may result in cell loss, the primary explant technique is preferred in such cases.

However, it has limitations, such as the poor adhesion of certain tissues to the growth surface and the selective outgrowth of cells. Nevertheless, this technique is effective for a wide range of embryonic cells, including fibroblasts, myoblasts, epithelial cells, and glial cells.

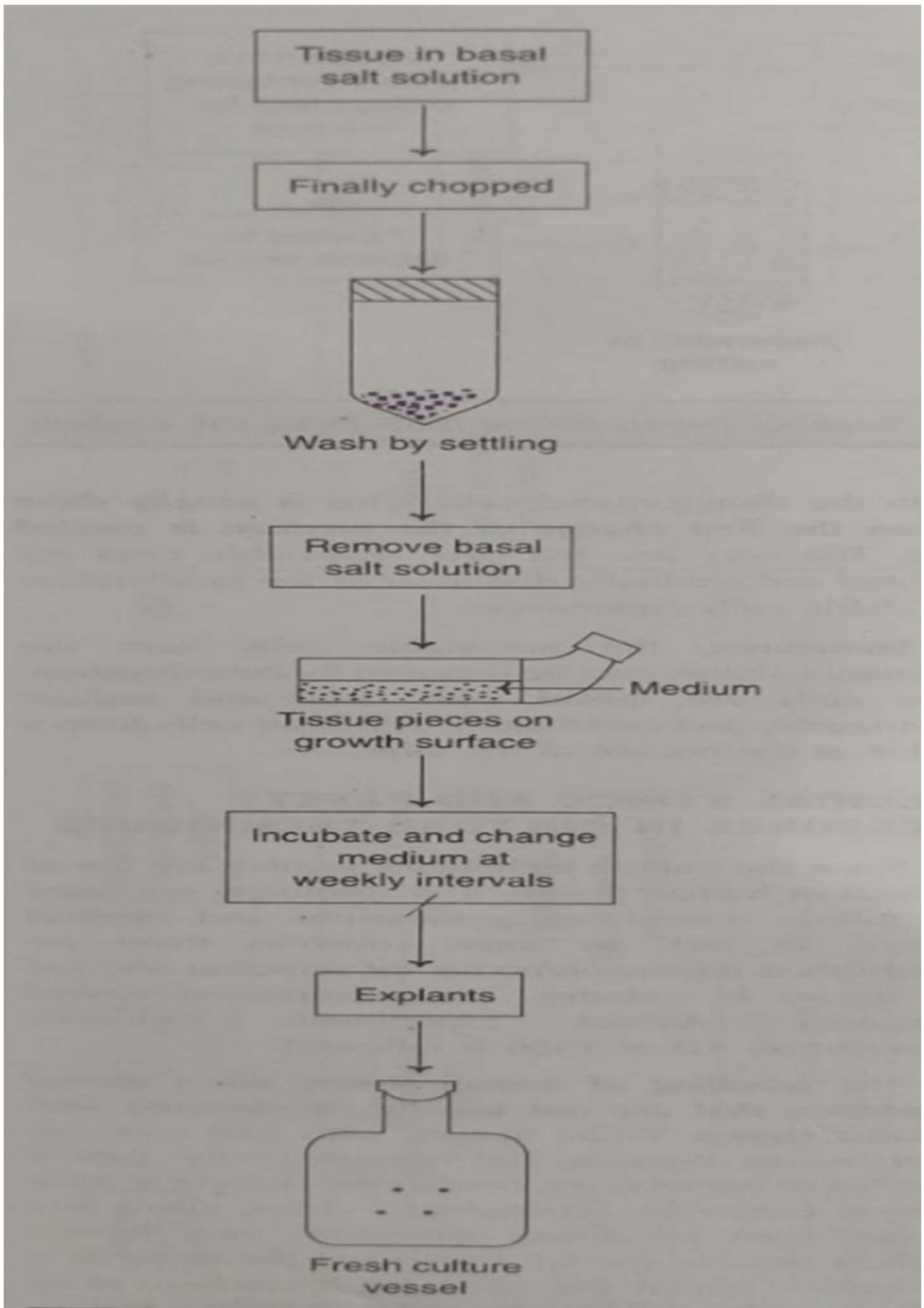

Fig. 36.4 : Primary explant technique for primary culture.

CELL LINES

After the initial establishment and development described above in primary cultures, the term "cell line" refers to the continued propagation of cultures following the first subculture. Essentially, once a primary culture undergoes subculture, it transitions into a cell line. A cell line typically consists of multiple cell lineages with either similar or distinct phenotypes.

Specific cell lineages can be selected through cloning, physical cell separation, or other methods of selection. When a cell line is derived through such processes, it is termed a "cell strain." Cell strains, however, do not have an indefinite lifespan and typically cease dividing after a certain number of divisions.

Finite Cell Lines

Cells in culture have a limited capacity for division before their growth rate diminishes and they eventually expire. These cell lines, which have a restricted lifespan in culture, are known as finite cell lines. Typically, cells divide between 20 to 100 times (equivalent to 20-100 population doublings) before they senesce. The exact number of doublings can vary depending on factors such as species, cell lineage, and culture conditions. For example, human cells generally divide 50-100 times, while murine cells divide 30-50 times before reaching the end of their lifespan.

Continuous Cell Lines

Occasionally, cells in culture undergo morphological changes and acquire altered characteristics that enable them to proliferate more rapidly, establishing an independent culture. The progeny derived from these altered cells have an unlimited lifespan compared to the original cell strains they were derived from. These are termed continuous cell lines. Continuous cell lines are often transformed, immortalized, and in some cases, tumorigenic. Transformation of cells into continuous cell lines can occur through treatment with chemical carcinogens or infection with oncogenic viruses.

Several commonly used terms in relation to cell lines are defined below:

- **Split Ratio:** The ratio used to dilute a cell culture during subculture. For example, a split ratio of 1:2 indicates that the culture is divided into half during each subculture.
- **Passage Number:** The number of times a culture has been sub cultured.
- **Generation Number:** The number of doublings that a cell population has undergone.

It's important to note that the passage number and generation number are distinct terms and refer to different aspects of cell culture history.

Commonly Used Cell Lines

Thousands of cell lines have been developed in laboratories worldwide. Table 36.2 provides a selected list of commonly used cell lines, detailing their origin, morphology, and other pertinent characteristics.

TABLE 36.2 A selected list of commonly used cell lines

Cell line	*Species of origin*	*Tissue of origin*	*Morphology*	*Ploidy*	*Characteristics*
IMR-90	Human	Lung	Fibroblast	Diploid	Susceptible to human viral infections.
3T3-A31	Mouse	Connective tissue	Fibroblast	Aneuploid	Contact inhibited, readily transformed
BHK21-C13	Hamster (Syrian)	Kidney	Fibroblast	Aneuploid	Readily transformable
CHO-k1	Chinese hamster	Ovary	Fibroblast	Diploid	Simple karyotype
NRK49F	Rat	Kidney	Fibroblast	Aneuploid	Induction of suspension growth by TGF-α, β.
BRL 3A	Rat	Liver	Epithelial	Diploid	Produces IGF-2
Vero	Monkey	Kidney	Fibroblast	Aneuploid	Viral substrate and assay
HeLa-S_3	Human	Cervical carcinoma	Epithelial	Aneuploid	Rapid growth, high plating efficiency.
Sk/HEP-I	Human	Hepatoma	Endothelial	Aneuploid	Factor VIII
Caco-2	Human	Colo-rectal carcinoma	Epithelial	Aneuploid with polarised	Forms tight monolayer support.
MCF-7	Human	Breast tumor (effusion)	Epithelial	Aneuploid	Estrogen receptor positive.
Friend	Mouse	Spleen	Suspension	Aneuploid	Hemoglobin, growth hormone.

SELECTION OF CELL LINES

When selecting a cell line, several factors need to be considered:

1. **Species:** Non-human cell lines are generally preferred due to lower biohazard risks. However, species differences must be considered when extrapolating data to humans.
2. **Finite or Continuous Cell Lines:** Continuous cell lines grow faster, are easier to clone and maintain, and produce higher yields. However, their appropriateness for expressing the correct functions of cells may be questionable, leading some to prefer finite cell lines despite the difficulties.
3. **Normal or Transformed Cells:** Transformed cells are preferred as they are immortal and grow rapidly.
4. **Availability:** The availability of cell lines is important, and sometimes it may be necessary to develop a particular cell line in the laboratory.
5. **Growth Characteristics:** Important parameters include population doubling time, ability to grow in suspension, saturation density (yield per flask), and cloning efficiency.
6. **Stability:** Stability with reference to cloning, generating adequate stock, and storage is important.
7. **Phenotypic Expression:** Cell lines must possess cells with the correct phenotypic expression.

Commonly used cell lines are developed in various laboratories worldwide, each with specific origins, morphologies, and characteristics.

Physical Requirements for Growing Animal Cells in Culture:

1. **Sterile Environment**: Maintaining sterility is crucial to prevent contamination by bacteria, fungi, or other microorganisms that can adversely affect cell growth.
2. **Temperature Control**: Cells are typically cultured at 37°C, which mimics the physiological conditions of the body for optimal growth and metabolism.
3. **pH Regulation**: Culture medium pH is maintained around 7.2 to 7.4 to ensure physiological conditions and prevent acidification or alkalization, which can impair cell viability.
4. **Oxygenation**: Adequate oxygen supply is essential as cells require oxygen for oxidative phosphorylation and energy production. This is usually achieved by ensuring proper ventilation and gas exchange in the incubator.
5. **Humidity Control**: Maintaining high humidity levels (around 95%) within the incubator helps prevent evaporation from the culture medium and maintains cell hydration.
6. **Nutrient Supply**: Cells require essential nutrients such as amino acids, vitamins, minerals, and glucose for growth. These are provided in the form of a balanced culture medium.
7. **Adherent Surface**: Some animal cells require a solid surface to attach and grow (e.g., glass or plastic). Surface treatments like polylysine or collagen coating may be necessary to enhance attachment.

8. **Incubation Conditions**: Cultures are typically maintained in CO_2 incubators with controlled atmospheric conditions (5% CO_2) to maintain pH balance in the medium and support cell growth.
9. **Lighting**: Continuous exposure to light is generally avoided as it can cause oxidative stress to cells. Incubators are typically kept in dark or low-light conditions.
10. **Monitoring and Maintenance**: Regular monitoring of culture conditions, pH, and cell morphology is essential. Medium changes and subcultures are performed at appropriate intervals to maintain healthy cell growth.

MODEL QUESTIONS (According to paper pattern)

Multiple Choice Questions (MCQs) with Answers

1. Which of the following is a method used for initiating cell culture?

- A) Microinjection
- B) PCR amplification
- C) Western blotting
- D) Mechanical disaggregation
  Answer: D) Mechanical disaggregation

2. What term describes the condition when cultured cells make close contact with each other, utilizing all available growth area?

- A) Differentiation
- B) Confluence
- C) Transformation
- D) Metastasis
  Answer: B) Confluence

3. Why are embryonic tissues preferred over adult tissues for primary cultures?

- A) Embryonic cells are easier to clone
- B) Adult tissues have higher cell viability
- C) Embryonic cells yield more viable cells and proliferate rapidly in vitro
- D) Adult tissues require fewer growth factors
  Answer: C) Embryonic cells yield more viable cells and proliferate rapidly in vitro

4. Which technique for primary culture involves finely chopping tissue pieces and spreading them over a growth surface?

- A) Mechanical disaggregation
- B) Enzymatic disaggregation
- C) Primary explant technique
- D) Microinjection
  Answer: C) Primary explant technique

5. What distinguishes finite cell lines from continuous cell lines?

- A) Finite cell lines are immortal, while continuous cell lines have limited lifespan
- B) Continuous cell lines are derived from transformed cells
- C) Finite cell lines are easier to clone
- D) Continuous cell lines grow faster and produce higher yields
  Answer: B) Continuous cell lines grow faster and produce higher yields

6. Which factor is NOT considered important when selecting a cell line for research?

- A) Species of origin
- B) Finite or continuous nature
- C) Growth characteristics
- D) Medium colour
  Answer: D) Medium colour

7. What is the preferred temperature for maintaining cell cultures in an incubator?

- A) 25°C
- B) 37°C
- C) 4°C
- D) 50°C
  Answer: B) 37°C

8. What role does foetal bovine serum play in cell culture medium?

- A) It provides antibiotics
- B) It promotes cell adhesion
- C) It enhances cell differentiation
- D) It supplies essential nutrients and growth factors
  Answer: D) It supplies essential nutrients and growth factors

9. Why is pH regulation important in cell culture?

- A) To prevent contamination
- B) To maintain sterility
- C) To ensure proper gas exchange
- D) To prevent acidification or alkalization that can harm cells
  Answer: D) To prevent acidification or alkalization that can harm cells

10. What is the purpose of maintaining high humidity in a cell culture incubator?

- A) To prevent microbial growth
- B) To maintain cell hydration
- C) To enhance cell differentiation
- D) To reduce CO_2 levels
  Answer: B) To maintain cell hydration

<u>Short Answer Questions (7 Marks, 300 words)</u>

1. Describe the process of initiating primary cell culture, highlighting the importance of embryonic tissues.

Answer:

Primary cell culture begins with the isolation of cells from tissues, which can be achieved through mechanical or enzymatic methods. Mechanical disaggregation involves physically

breaking down tissues, while enzymatic disaggregation uses enzymes to dissociate cells. Once isolated, cells are cultured under controlled conditions before the first subculture.

Embryonic tissues are preferred over adult tissues for primary cultures due to several advantages. Firstly, embryonic cells are easier to disaggregate, yielding more viable cells that proliferate rapidly in vitro. This characteristic is crucial for establishing primary cultures from small tissue masses. Secondly, embryonic cells have a higher capacity for proliferation compared to adult cells, which may senesce more quickly in culture. Thirdly, embryonic cells retain a greater potential for differentiation, allowing researchers to study various cell types within the same culture.

The process of initiating primary cell culture also involves optimizing culture conditions such as medium composition, pH, and temperature. A nutrient-rich medium supplemented with foetal bovine serum is commonly used to support cell growth and maintain viability. pH regulation is critical to prevent acidification or alkalization, which can compromise cell health. Temperature control, typically at 37°C, mimics physiological conditions necessary for optimal cell metabolism and growth.

So, initiating primary cell culture involves selecting appropriate tissue sources, optimizing isolation techniques, and maintaining optimal culture conditions. Embryonic tissues offer distinct advantages in this process, facilitating the establishment of diverse and viable primary cultures for research and experimental purposes.

2. Compare and contrast finite cell lines with continuous cell lines, discussing their applications and limitations in cell culture research.

Answer:

Finite cell lines and continuous cell lines represent two distinct types of cultured cells, each with unique characteristics and applications in cell culture research.

Finite Cell Lines: Finite cell lines have a limited lifespan and can only undergo a finite number of cell divisions before senescence and eventual cell death. This lifespan typically ranges from 20 to 100 population doublings, depending on the species, cell type, and culture conditions. Human cells, for example, often divide approximately 50 to 100 times before reaching senescence. Finite cell lines are derived directly from primary cultures or through subculturing primary cells, retaining many of the differentiated characteristics of the original tissue.

Applications of finite cell lines include:

- Studying short-term cellular responses to experimental treatments.
- Investigating specific biological processes within a defined timeframe.
- Serving as models for understanding cellular senescence and aging-related mechanisms.

Limitations of finite cell lines include:

- Inability to maintain continuous proliferation, limiting long-term studies.
- Variability in lifespan among different cell types, complicating experimental consistency.
- Challenges in maintaining genetic stability over multiple passages.

Continuous Cell Lines: Continuous cell lines, in contrast, are immortalized and can proliferate indefinitely under appropriate culture conditions. These cell lines are often derived from primary cultures or finite cell lines that have been transformed or immortalized through genetic modification, viral infection, or exposure to chemical agents. Continuous cell lines typically exhibit altered growth characteristics, such as increased proliferation rate and morphological changes compared to their primary counterparts.

Applications of continuous cell lines include:

- Producing large quantities of cells for commercial and research purposes.
- Studying long-term cellular processes, including carcinogenesis and tumour progression.
- Serving as robust models for drug screening and therapeutic development.

Limitations of continuous cell lines include:

- Potential loss of differentiated functions observed in primary cells.
- Genetic and phenotypic changes due to immortalization or transformation processes.
- Ethical concerns related to the use of transformed cells in research and testing.

In conclusion, the choice between finite and continuous cell lines depends on the specific research objectives, experimental design, and ethical considerations. Both types of cell lines play critical roles in advancing our understanding of cellular biology, disease mechanisms, and therapeutic interventions in biomedical research.

3. Discuss the importance of primary culture techniques in cell biology research, emphasizing the advantages of each method (mechanical disaggregation, enzymatic disaggregation, and primary explant technique) and their applications.

Answer:

Importance of Primary Culture Techniques in Cell Biology Research:

Primary culture techniques are crucial in cell biology research as they facilitate the direct study of cells derived from tissues under controlled laboratory conditions. These techniques offer several advantages over established cell lines, including the preservation of tissue architecture, retention of tissue-specific functions, and the ability to study primary cells in their natural environment.

Advantages and Applications of Mechanical Disaggregation:

Mechanical disaggregation involves physically breaking down tissues into smaller fragments without the use of enzymes. This method is advantageous for preserving cell surface markers and morphology, making it suitable for isolating specific cell types based on size and shape. Mechanical disaggregation finds applications in culturing skeletal muscle cells, adipocytes, and fibroblasts from connective tissues, where maintaining cellular integrity is critical for accurate functional studies.

Advantages and Applications of Enzymatic Disaggregation:

Enzymatic disaggregation utilizes proteolytic enzymes like trypsin or collagenase to degrade extracellular matrix components and dissociate cells from tissue structures. This method is highly efficient in releasing cells while preserving cell viability and functionality. Enzymatic

disaggregation is preferred for tissues with dense matrices or complex cellular architectures, such as isolating hepatocytes from liver tissues or preparing neuronal cultures for neurobiology research. It enables researchers to study cell interactions and responses within tissue-specific microenvironments.

Advantages and Applications of Primary Explant Technique:

The primary explant technique involves culturing small tissue fragments (explants) directly onto a culture surface and allowing cells to migrate and proliferate from the explant. This method preserves tissue architecture and cellular interactions, mimicking the natural environment of cells within tissues. The primary explant technique is particularly useful for tissues that do not disaggregate easily or for studying complex cellular interactions within intact tissue structures. Applications include culturing skin biopsies for dermatological research, cartilage explants for orthopaedic studies, and neural tissue explants for neurobiology research, facilitating the study of tissue-specific functions and disease mechanisms.

Long Answer Questions (10 Marks, 500 words)

Discuss the essential physical requirements for growing animal cells in culture, emphasizing the importance of maintaining optimal conditions for cell viability, growth, and experimental reproducibility. Provide detailed insights into each requirement, their implications on cell behaviour, and practical considerations in laboratory settings.

Answer:

Introduction: Growing animal cells in culture is a fundamental technique in biological research, biotechnology, and pharmaceutical development. The success of cell culture experiments hinges upon maintaining precise physical conditions that mimic the physiological environment of cells in vivo. This ensures optimal cell viability, growth, and the ability to study cellular processes under controlled conditions.

Physical Requirements for Growing Animal Cells in Culture:

1. **Sterile Environment:**
    - **Importance:** Maintaining a sterile environment is paramount to prevent contamination by bacteria, fungi, or other microorganisms, which can compromise cell health and experimental integrity.
    - **Implications:** Contamination can lead to inaccurate experimental results, cell death, or unwanted cellular interactions that skew research outcomes.
    - **Practical Considerations:** Use of laminar flow hoods, autoclaving of equipment and media, and strict aseptic techniques are essential for maintaining sterility.
2. **Temperature Control:**
    - **Importance:** Cells are typically cultured at 37°C, mirroring physiological body temperature, to ensure optimal enzymatic activity, metabolic function, and growth.
    - **Implications:** Temperature deviations can alter cellular metabolism, protein synthesis rates, and overall cell behaviour, affecting experimental reproducibility.

- **Practical Considerations:** Use of CO_2 incubators with precise temperature control and regular monitoring of incubator settings to maintain stable culture conditions.

3. **pH Regulation:**
   - **Importance:** Culture medium pH is maintained around 7.2 to 7.4 to mimic physiological conditions and support cellular functions such as enzyme activity and ion transport.
   - **Implications:** pH shifts can affect cell viability, growth rates, and metabolism, leading to cell stress or death.
   - **Practical Considerations:** Regular pH monitoring with pH meters, use of CO_2 incubators to regulate CO_2 levels and buffer systems in media to stabilize pH.
4. **Oxygenation:**
   - **Importance:** Adequate oxygen supply is crucial for cellular respiration, energy production through oxidative phosphorylation, and maintaining redox balance.
   - **Implications:** Oxygen deprivation (hypoxia) or excess can alter cellular metabolism, induce oxidative stress, and affect cell survival.
   - **Practical Considerations:** Proper ventilation in incubators, adjusting gas exchange rates, and monitoring dissolved oxygen levels in culture media.
5. **Humidity Control:**
   - **Importance:** High humidity levels (around 95%) within the incubator prevent evaporation from the culture medium, maintain cell hydration, and prevent osmotic stress.
   - **Implications:** Inadequate humidity can lead to media evaporation, cellular desiccation, and compromised cell health.
   - **Practical Considerations:** Use of humidified CO_2 incubators, regular monitoring of humidity levels, and maintaining water reservoirs within incubators.
6. **Nutrient Supply:**
   - **Importance:** Cells require essential nutrients such as amino acids, vitamins, minerals, and glucose for growth, metabolism, and maintaining cellular functions.
   - **Implications:** Inadequate nutrient supply can impair cell growth, viability, and alter cellular phenotype and behaviour.
   - **Practical Considerations:** Preparation of nutrient-rich culture media, supplementation with serum or growth factors, and regular media changes to ensure nutrient availability.
7. **Adherent Surface:**
   - **Importance:** Some animal cells require a solid surface (e.g., glass or plastic) coated with extracellular matrix components (e.g., collagen, fibronectin) for attachment and growth.
   - **Implications:** Improper surface treatment or coating can affect cell adhesion, spreading, and morphology.
   - **Practical Considerations:** Pre-coating culture vessels with appropriate matrices, optimizing coating concentrations, and evaluating surface adhesion efficiency.
8. **Incubation Conditions:**

- **Importance:** Cultures are maintained in CO_2 incubators with controlled atmospheric conditions (typically 5% CO_2) to stabilize pH and support cellular metabolism.
- **Implications:** Inconsistent incubator conditions can impact cell growth rates, metabolism, and alter experimental outcomes.
- **Practical Considerations:** Regular calibration of CO_2 levels, monitoring of temperature, humidity, and gas exchange rates, and ensuring proper function of incubator sensors and alarms.

9. **Lighting:**
   - **Importance:** Continuous exposure to light can induce oxidative stress in cells and alter cellular responses in experiments.
   - **Implications:** Light-sensitive experiments require incubators maintained in dark or low-light conditions to prevent phototoxicity.
   - **Practical Considerations:** Use of light-shielded incubators, covering culture vessels with light-blocking materials, and minimizing exposure to ambient light.
10. **Monitoring and Maintenance:**
    - **Importance:** Regular monitoring of culture conditions, pH, cell morphology, and media changes are critical for maintaining healthy cell cultures and experimental reproducibility.
    - **Implications:** Neglecting routine maintenance can lead to suboptimal culture conditions, contamination, or cell death.
    - **Practical Considerations:** Establishing regular culture maintenance schedules, documenting culture conditions, and implementing quality control measures to ensure consistent experimental outcomes.

The physical requirements for growing animal cells in culture are essential for maintaining optimal cell viability, growth, and experimental reproducibility. Each requirement plays a critical role in creating a conducive environment that mimics physiological conditions, facilitating accurate study of cellular behaviours, disease mechanisms, and therapeutic interventions in vitro. Adherence to stringent culture practices and continuous improvement of techniques are crucial for advancing cell culture research and applications in biomedical sciences.