الكلية	العلوم
القسم	علوم الحياة
المادة باللغة الانجليزية	Biotechnology and genetic engineering
المادة باللغة العربية	التقنيات الاحيائية والهندسة الوراثية
المرحلة الدراسية	الرابعة
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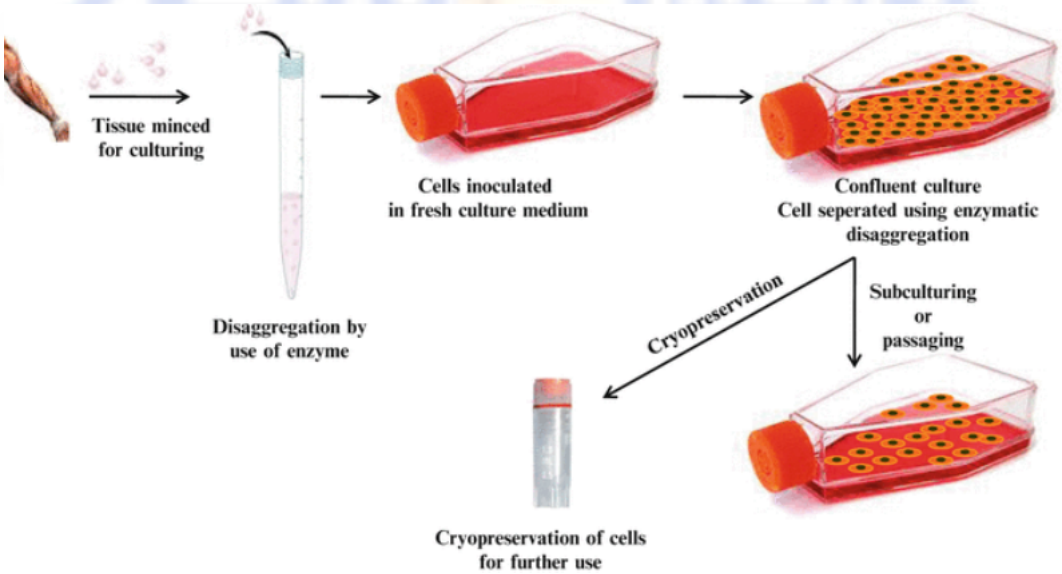
عنوان المحاضرة باللغة الانجليزية	Plant and animal tissue cultures
عنوان المحاضرة باللغة العربية	المزارع النسيجية النباتية والحيوانية
رقم المحاضرة	الثانية
المصادر والمراجع	Jones and Bartlett Publishers, (October 20, 2010) Introduction to Biotechnology and Genetic Engineering, 1st Edition.
	Gao, J. (2012). Tissue culture and rapid multiplication techniques of Apocynum L. Agricultural Science & Technology, 13(11), 2269.

Disadvantages of Plant Tissue culture:

- It involves more labor and cost more money in building the facility and equipping the lab with all the instruments and chemicals.
- There is a chance that the propagated plants will be less resilient to diseases when grown in outside conditions due to the type of environment they are grown in.
- It is essential that, before being cultured, the material is screened; failure to pick up any abnormalities could lead to the new plants being infected.
- While the success rate is high if the correct procedures are followed, success with the tissue culture is not a guarantee. That's why accurate protocols are necessary to grow plants in tissue culture setting, which can be laborious when you try to create one working protocol by yourself.
- Contamination is the major issue in tissue culture setting. Plants can get infected by bacteria, fungi, and viruses. That's why all measures should be taken and PPE kit should be used while performing tissue culture in your lab.
- Tissue culture is an advanced technique and require some advanced knowledge and practice for anyone to get started in the field.

What is Cell and Tissue Culture?

Tissue Culture is the general term for the removal of cells, tissues, or organs from an animal or plant and their subsequent placement into an artificial environment conducive to growth. This environment usually consists of a suitable glass or plastic culture vessel containing a liquid or semi-solid medium that supplies the nutrients essential for survival and growth. The culture of whole organs or intact organ fragments with the intent of studying their continued function or development is called **Organ Culture**. When the cells are removed from the organ fragments prior to, or during cultivation, thus disrupting their normal relation- ships with neighbouring cells, it is called **Cell Culture**. Although animal cell culture was first successfully under taken by Ross Harrison in 1907, it was not until the late 1940's to early 1950's that several developments occurred that made cell culture widely available as a tool for scientists. For example, there was the development of antibiotics that made it



easier to avoid many of the contamination problems that plagued earlier cell culture attempts.

Types of cell culture:

1- Primary cell culture:

These cells are obtained directly from tissues and organs by mechanical or chemical disintegration or by enzymatic digestion. These cells are induced to grow in suitable glass or plastic containers with complex media. These cultures usually have a low growth rate and are heterogeneous; however, they are still preferred over cell lines as these are more representative of the cell types in the tissues from which they are derived.

2- Secondary cell culture:

When primary cell cultures are passaged or subcultured and grown for a long period of time in fresh medium, they form secondary cultures and are long-lasting (unlike cells of primary cell cultures) due to the availability of fresh nutrients at regular intervals. The passaging or subculturing is carried out by enzymatic digestion of adherent cells. This is followed by washing and re-suspending of the required amount of cells in appropriate volumes of growth media. Secondary cell cultures are preferred as these are easy to grow and are readily available; they have been useful in virological, immunological, and toxicological research.

According to the life span of culture, the cell lines are categorized into two types:

- Finite cell lines

These cell lines are known to have limited number of cell division during their life span. The cells passage several times and then lose their ability to proliferate, which is a genetically determined event known as senescence. Cell lines derived from primary cultures of normal cells are finite cell lines.

- Continuous cell lines

When a finite cell line undergoes transformation and gains the ability to divide indefinitely, it becomes a continuous cell line. Such transformation/mutation can occur spontaneously or can be chemically or virally induced or from the establishment of cell cultures from malignant tissue.

Quantitation:

Quantitation is carried out to characterize cell growth and to establish reproducible culture conditions.

Hemocytometer

Cell counts are important for monitoring growth rates as well as for setting up new cultures with known cell numbers. The most widely used type of counting chamber is called a hemocytometer. It is used to estimate cell number. The concentration of cells in suspension is determined by placing the cells in an optically clear chamber under a microscope. The cell number within a defined area of known depth is counted, and the cell concentration is determined from the count.

Electronic counting

For high-throughput work, electronic cell counters are used to determine the concentration of each sample.

Other quantitation

In some cases, the DNA content or the protein concentration needs to be determined instead of the number of cells.

Growth Requirements

The culture media used for cell cultures are generally quite complex, and culture condition widely varies for each cell type. However, media generally include amino acids (source of nitrogen), vitamins (cofactors), salts (maintain osmotic pressure), glucose (source of energy, carbon,) a bicarbonate buffer system (maintains a pH between 7.2 and 7.4), growth factors and hormones (growth stimulators), O2 and CO2. To obtain best growth, addition of a small amount of blood serum is usually necessary, and several antibiotics, like penicillin and streptomycin are added to prevent bacterial contamination. Temperature varies on the type of host cell. Most mammalian cells are maintained at 37°C for optimal growth, while cells derived from cold- blooded animals tolerate a wider temperature range (i.e., 15°C to 26°C).

Advantages of animal cell culture

1- Physiochemical and physiological condition: Role and effect of pH, temperature, O2/CO2 concentration, and osmotic pressure of the culture media can be altered to study their effects on the cell culture.
2- Metabolism of cell: To study cell metabolism and investigate the physiology and biochemistry of cells.
3- Cytotoxic assay: Effect of various compounds or drugs on specific cell types such as liver cells can be studied.

4- Homogenous cultures: These cultures help study the biology and origin of the cells.
5- Valuable biological data from large-scale cell cultures: Specific proteins can be synthesized in large quantities from genetically modified cells in large-scale cultures.
6- Consistency of results: Reproducibility of the results that can be obtained by the use of a single type/clonal population.
7- Identification of cell type: Specific cell types can be detected by the presence of markers such as molecules or by karyotyping.
8- Vaccines production, Virus cultivation and study, Gene therapy, Cancer research, Cellular and molecular study.

Disadvantages of animal cell culture

1- Expenditure and expertise: This is a specialized technique that requires aseptic conditions, trained personnel, and costly equipment.
2- Dedifferentiation: Cell characteristics can change after a period of continuous growth of cells in cultures, leading to differentiated properties compared to the original strain.
3- Low amount of product: The miniscule amount of mAB and recombinant protein produced followed by downstream processing for extracting pure products increases expenses tremendously.
4- Contamination: Mycoplasma and viral infection are difficult to detect and are highly contagious.
5- Instability: Aneuploidy chromosomal constitution in continuous cell lines leads to instability. In addition, this system cannot replace the complex live animal for testing the response of chemicals or the impact of vaccines or toxins.