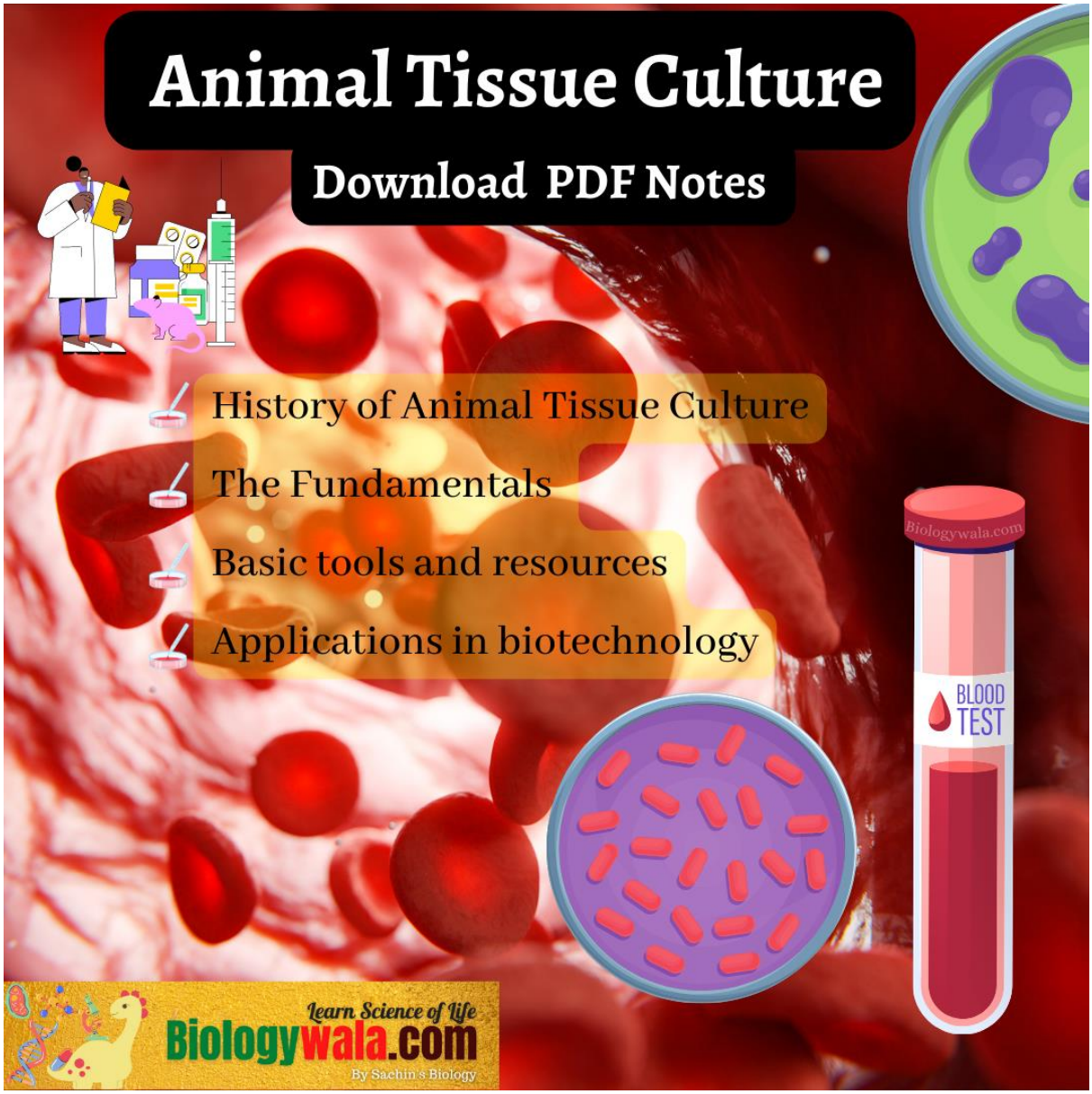
Animal Tissue Culture: History, Fundamentals, Roles and Requirements



INTRODUCTION

Animal tissue culture, which involves the cultivation of cells derived from animals, plays a significant role in understanding multicellularity—a fundamental trait shared by the majority of organisms. Multicellularity enables cells to specialize, allowing them to focus on specific tasks required for the organism's survival. In higher organisms, while cells generally share organelles and metabolic processes, they also exhibit individual variations in the expression of these elements to fulfil distinct purposes within the organism.

Because of this cellular specialization, animals are made up of a variety of different cell types, each of which has a unique size, shape, structure, and function. These cells are said to as differentiating. More than 100 different cell types can be found in a vertebrate. These cells joined together in highly structured patterns to carry out certain tasks.

With the right techniques, many animal cells can be made to proliferate outside of the organ or tissue they originated from. When incubated at specific temperatures in an incubator and supplemented with a solution containing cell nutrients and growth factors, isolated cells, tissues, or organs can be grown in plastic dishes.

The list of cell types that can be cultivated in culture includes epithelial tissue, skeletal, cardiac, and smooth muscle cells, as well as connective tissue components like fibroblasts and bone and cartilage (liver, lung, breast, skin, bladder and kidney), Melanocytes, endocrine cells (pituitary, adrenal, pancreatic islet cells), neural cells (glial cells and neurons, but neurons do not multiply in vitro), and several tumour cell types. These tissue culture techniques owe a lot to two important fields of medical study: virology and cancer research.

History of Animal Tissue Culture:

Animal Tissue Culture is not a new method; references to its usage in the scientific literature date back to 1885. The medullary plate of a chick embryo was able to survive for a few days in warm saline thanks to an embryologist by the name of Roux. The first instance of a successful explanation was this one. Jolly performed thorough observations in 1903 utilizing salamander leukocytes regarding in vitro cell survival and cell division. The term "tissue culture" originated from early investigations that explored tissue pieces. When cells are kept in vitro for more than 24 hours, the term tissue culture is used formally.

The discovery of cell entrapment and growth from explants of frog embryo tissue by Ross Harrison in 1907 is credited with the advancement of animal cell culture. Animal cell culture as a science is frequently seen as having its roots in the cell growth that he discovered in clotted lymph fluid on a depression slide.

His method, known as "the hanging drop," involved suspending the separated tissue on the underside of a coverslip that was placed over a depression in a microscope slide. Burrows continued to refine this method by using plasma clots, which were discovered to be more effective for the proliferation of warm-blooded animal cell lines.

The Fundamentals of Animal Tissue Culture

Culturing of tissue

The growing of organs, tissues, and cells in vitro is referred to as tissue culture. The term originally included the in vitro culture of plant cells as well as animal cells. Organ culture, explant culture, and cell culture are the three main subcategories of Animal Tissue Culture.

Organ cultivation

An organ is defined as a three-dimensional culture of tissue that retains all or some of the histological characteristics of the tissue in vivo. By cultivating the tissue at the liquid-gas interface on a grid or gel, the entire organ or a portion of it is kept in a way that allows differentiation and preservation of architecture.

In Animal Tissue Culture, the culture of organs has drawbacks. As tissues cannot be multiplied into new organs, each one can only be utilized once, it is challenging to determine whether a reaction is reproducible. Also, it's possible that a given piece of tissue only has a very tiny number of the specific cells of interest, making it challenging to identify and measure the reaction.

Because the tissue lacks a functional circulatory system, it may be impossible to provide enough oxygen and nutrients to all parts of it, leading to some cells dying quite quickly. By storing the organ in stirred cultures or in roller bottles that alternately supply air and soluble nutrients, this issue may be somewhat alleviated.

Explants culture

Explant culture is a technique in which tiny fragments of the desired tissue are simply allowed to adhere to a suitable substrate, typically one that has been coated with collagen and is then grown in a rich medium, typically one containing serum. Cell migration is encouraged in the plane of the solid substrate after attachment. Explants are often kept in Maximov chambers, which are still in use today.

In these chambers, cells are grown on coverslips that are sealed over a depression in a thick glass slide. Regular culture dishes are now frequently used, which is considerably more convenient because they do not need to be disassembled and rebuilt at every feeding.

(Dissociated) Culture of cells

Cell culture is the term used to describe cultures made from primary cell cultures, or dissociated cells extracted from the source tissue in Animal Tissue Culture. It is possible to culture cells as a monolayer on a solid substrate or as a suspension in the culture medium after the cells have been physically and/or enzymatically dispersed into a cell suspension.

These Animal Tissue Cultures no longer possess the historic architecture and frequently some of the related metabolic characteristics. They may, however, be spread, multiplied, and divided to create duplicate cultures.

It is possible to describe cell cultures and freeze a specific population to preserve it.

Basic tools and resources for animal cell culture

For the upkeep of cell cultures, some specialized tools and methods are needed. As a general rule, you can execute cell culturing more effectively the more equipment you have.

Sterile workspace

A separate space should be made accessible whenever possible for clean cell culture activity.

If at all possible, this space should have an airflow cabinet that circulates filtered air around the desk and be free of traffic. Although desirable, a HEPA (High-Efficiency Particle Air Filter) filtered air supply is not always feasible. The cell culture laboratory must be expressly designated for clean cell culture activity, and no primary animal tissue or microorganisms may be cultivated there or close by.

Clean lab coats should not be worn outside of this lab and brought back inside; instead, they should be left at the entrance. A laminar flow hood provides the finest sterile protection available if rigorous sterility is required. A Class II Biohazard Cabinet with a vertical laminar flow should be used while handling dangerous chemicals.

Nonetheless, a designated location for sterile work should be set aside where there is no route for primary cultures and if a laminar flow hood or sterile room is not accessible. Sterility can be easily maintained if aseptic procedures are followed and the space is kept clean and organized.

Incubation facilities

To keep the cells at 30–40 °C, the cell culture laboratory will need to be equipped with an incubator or hot chamber in addition to an airflow cabinet and readily cleanable benching. The type of cells being grown will determine the incubation temperature.

Mammalian cells need a temperature of 37 °C, but insect cells will develop best at a temperature of about 30 °C. To maintain an environment of between 2-5% CO2, it might be essential to employ an incubator that has been built to allow CO2 to be supplied from a main supply or gas cylinder.

Freezers and refrigerators (-20 °C)

Both of these things are crucial for the storage of liquid media at 4 °C, as well as for some media components (such as glutamine and serum) and enzymes (such as trypsin) at -20 °C. For the storage of medium and buffers, a refrigerator or cold room is necessary.

For storing pre-aliquoted stocks of serum, nutrition, and antibiotics, a freezer will be required. Reagents can be kept at a temperature of -20 °C, but if cells are to be preserved, liquid nitrogen or a freezer set at -70 °C may be required.

Microscopes

It is necessary to use a basic inverted microscope to study cultures in flasks and dishes. Understanding morphological changes in cultures is essential since they may be the earliest sign of a culture's decline. For ordinary hemocytometer cell counts, a fairly basic light microscope with x100 magnification will do; however, chromosome analysis or autoradiography operations will require a microscope of a much higher grade.

Tissue culture ware

There are several different types of tissue culture plasticware available, with specially treated polystyrene being the most popular. Even if every plasticware used in tissue culture should be able to sustain cell development adequately, it is crucial to check that cultures can thrive in a new dish or provider. The techniques to verify this, like growth curves and the amount of time it takes to reach a confluent monolayer, are comparable to those used to verify that batches of serum are sufficient.

Petri plates or flasks (25 cm^2 or 75 cm^2) can be used to sustain cells, and the flasks have the added benefit of being able to be gassed before being sealed, eliminating the need for a CO_2 incubator. This is especially helpful if incubators malfunction. Always select tissue culture equipment by the method.

Facilities for cleaning and sterilization

Wide selections of plastic tissue cultures are readily available, which decreases the amount of cleanup required. Nonetheless, glass items like pipettes should be thoroughly washed with distilled water after being thoroughly immersed in an appropriate detergent, and before being dried and sterilized. Before placing pipettes into containers for sterilization, nonabsorbent cotton wool is frequently used as a stopper.

N_2 gas and a deep freezer

Inevitably, samples of cultures need to be frozen down for storage for continuous and finite cell lines. To avoid genetic drift and to protect against the loss of the cell line due to contamination and other calamities, it is crucial to maintain continuity in cells. For all cells in the culture, the process for freezing cells is the same.

They should be preserved with an appropriate preservative, often dimethylsulfoxide, and frozen in the exponential phase of growth (DMSO). The cells are stored either at -196 °C immersed in liquid N2 (in sealed glass ampoules) or above the liquid surface in the gas phase. The freezing is done gradually at 1 °C/min to -50 °C (screw top ampoules).

Device for reverse osmosis

For media preparation and glassware cleaning, a supply of water that has undergone reverse osmosis or double distillation is required. The pH of the double-distilled water should be monitored frequently because it occasionally varies. Water from a single source should be utilized since variations in the quality of the water used could explain variations in results. Autoclaving water for 20 minutes at 121 °C sterilizes it. If the distilled water is going to be used for making media, it needs to be made in glass and preserved in glass. Leaching of harmful compounds from plastic during storage may cause them to end up in the water.

Filter sterilization

Media that cannot be autoclaved must be sanitized using a membrane filter with a pore size of 0.22 m. They are available in a variety of designs to enable the filtration of a wide range of volumes (e.g., Millipore, Gelman). These can be sterilized by autoclaving in the appropriate filter holders or purchased as sterile, disposable filters.

Facilities for cell counting

Confluence can be used to visually track cell proliferation, however, for the majority of experimental uses, more precise cell counts are needed.

The Improved Neubauer hemocytometer, which was initially created for counting blood cells, is the most often used instrument. It comprises a slide that has been thickened and has a known-depth centre chamber. The bottom of the silvered chamber has a grid engraved into it. A suspension of individual cells is poured into the counting chamber in preparation for counting. To disperse cell clumps that are challenging to count precisely, it is crucial to properly aspirate the cell solution before loading the chamber.

Embryonic or adult tissues

Embryonic or adult tissue can be used to create cultures. In general, cultures made from embryonic tissue survive and develop more successfully than those made from adult tissue. Almost all embryonic tissue types are simple to cultivate, whereas adult tissues are frequently challenging or impossible to do so.

This most likely illustrates the embryo's lower level of specialization and the presence of replicating precursor or stem cells. Mature tissues frequently contain a more organized extracellular matrix that is less easily disaggregated and typically has a smaller growth fraction and a higher number of non-replicating specialized cells. It is more challenging to initiate and spread culture, and it frequently has a short lifespan.

selecting the animal cell culture categories

Early attempts to cultivate tissues relied on the explantation of complete organs or tissues that could only be kept in vitro for relatively brief periods. Although growing certain cell types from tissues is more common nowadays, there are still some circumstances where it is required to create an entire organ (or a part of it).

Adherent or suspension culture

Either in suspension or as an adhesive monolayer, cells can grow. Adherent cells are thought to be anchorage-dependent, and proliferating requires adhesion to a substrate. They typically experience contact inhibition, which means that they expand as an adhering monolayer and cease to divide when their density is such that they touch.

The majority of cells develop in this manner, except altered cells and mature hemopoietic cells. As subculture just needs to be diluted with media, suspension cultures are simpler to propagate. A proteolytic enzyme must be applied to cultures where cells bind to the substrate or one another to dissolve the bond between the two. Trypsin is the enzyme that is employed the most frequently, while other enzymes like collagenase, papain, dispase, and pronase are also utilized. Trypsinization is not necessary for freely suspended cultures. Hence, they are also simpler to harvest.

Continuous cell line or primary cell culture

A sequence of distinctive events takes place when you remove tissue from an embryo, separate it into a suspension of single cells, and plate them out onto a culture dish. First, cells go through a lag period, often lasting no longer than 1-2 days, during which there is little to no growth in the number of cells. Cells are "conditioning" the medium at this period by changing their internal cytoskeletal structure and enzyme composition as well as adapting to the new medium.

Second, the cells go through a time of quick division known as log phase growth. Because they are made from cells taken straight from the animal, primary cultures like the ones just described are what they are called. The cells develop, undergo division or not (depending on what they are used to), and eventually perish. It's back to the animal for the following experiment to gather fresh tissue and make fresh cultures.

Primary cultures: advantages and drawbacks

A primary culture is one that was "initiated from cells, tissues, or organs obtained directly from organisms," as was previously stated. The fundamental benefits of primary cultures include the preservation of

1) tissue-specific activities and

(2) the ability to undergo biotransformation.

The metabolic profile produced by primary cell cultures, such as hepatocytes, frequently resembles in vivo more closely than the pattern produced by exogenously supplied subcellular fractions. Hepatocytes have the capacity for conjugation processes that subcellular fractions could lack.

In actuality, in vivo products that were previously undetectable have been discovered as a result of metabolic research in hepatocytes. Ketoethinimate was shown to be an ethinimate metabolite through research on hepatocytes. Further research showed that this metabolite was produced in vivo as well.

The preservation of tissue-specific activities is the second benefit of primary cultures. For instance, synchronously beating primary cultures of rat cardiac cells can be created. Arrhythmias and heart-stopping were seen in these cultures after exposure to cardiotoxic tricyclic antidepressants. Primary cultures have the drawback of requiring cell isolation for every experiment.

The tissue must be broken down during cell isolation procedures, frequently using proteolytic enzymes. This could cause the integrity of the membrane to be compromised, the loss of cellular byproducts, and the loss or destruction of particular membrane receptors. Damage is frequently fixed while waiting for monolayer cultures to form. The loss of Ca++ during the separation of hepatocytes is an illustration of this phenomenon.

Continuous cell lines: benefits and drawbacks

When beginning any experimental investigation using a cell line, it is crucial to keep the following things in mind:

1. Some cell lines have been around for a long time and have gone through numerous in vitro iterations. Before usage, cultures must be thoroughly inspected for cell type homogeneity and, if necessary, re-cloned. Compared to newly separated short-term cultures, cell lines are often more resilient to toxic stress.
2. Since population doubling time may affect sensitivity to the test agents (i.e., cells may need to go through mitosis to reflect medication impact), researchers should

be aware of it. The number of cells seeded to achieve the requisite density may also be impacted by replication time.

3. The specific assay at hand should be best served by the type of cell and culture conditions used at the time of exposure to the test agent. Sparsely seeded cultures maintained over several days may be ideal for some experimental procedures. When cells are undergoing exponential growth or are confluent, other experiments may be more illuminating.
4. It is important to take notice of any normal or tumorigenic traits. Chromosome count and the capacity to replicate in media with low serum concentrations may also be indicative of this. (Low serum medium allows tumour cells to grow more quickly.)
5. Certain cell lines release or maintain tissue-specific proteins and enzymes that can be employed as biological markers in research. It is important to take note of certain biomarkers, such as unique receptor sites or reactions to particular growth hormones.

Applications of animal tissue culture in Biotechnology

One of the most crucial tools for scientific and therapeutic research has been animal cell culture. This article explores 9 uses for animal cell culture. Model Systems, Toxicity Testing, Cell-Based Manufacturing, Drug Screening and Development, Cancer Research, Virology, Genetic Engineering, Gene Therapy, and Stem Cell Therapy are just a few of the fields included in this list.

1) Design Systems

An excellent model system for understanding fundamental cell biology and metabolism is animal cell culture. Animal cell culture has been utilized in research as 2D and 3D culture models for a variety of experiments involving the study of drugs or infectious agents.

2) Toxicity Evaluation

Animal cell cultures are frequently employed to circumvent the use of animals in the testing of new medications, chemicals, and cosmetics for toxicity. Kidney and liver-derived cell cultures are the principal animal cell cultures employed in this field.

3) Cell-based Production

Viral generation on a big scale for vaccine manufacture is possible using animal cell cultures. This method has been applied to numerous vaccines, including those for hepatitis B, measles, polio, rabies, chickenpox, and mumps.

Animal cell culture can be utilized to create genetically engineered goods with commercial and medicinal applications in addition to producing viruses. Products of many kinds include insulin, hormones, and monoclonal antibodies.

4) Drug Development and Screening

Animal cell culture-based assays have grown in importance within the pharmaceutical sector. They are utilized for high-throughput drug screening in addition to toxicity testing. Read this post at kosheeka.com/primary-cells-a-valuable-in-vitro-model-for-drug-toxicity-studies/ for more information.

5) Research on cancer

In oncology research, animal cell cultures have been used for biomarker and molecular analyses. Moreover, various anticancer substances can be tested on cancer cells in culture. Current research in cancer hopes to eliminate cancer cells in a population that also contains normal primary cells.

6) Virology

In order to avoid using animal models, viruses have been replicated in animal cells. These reproduced viruses can be employed to isolate and study fundamental viruses or to produce vaccines.

7) Genetic engineering

Reprogramming genes to create new proteins is the core concept of genetic engineering. The capacity to introduce new genetic material into cells is known as transfection. To create novel proteins in high numbers for clinical research or therapeutic applications, animal cell cultures can be transfected.

8) Gene therapy

In addition to being utilized for genetic engineering, animal cell cultures can also be used for therapeutic purposes. A patient's cells can be taken out and replaced with created cells that have the required functional gene.

In conclusion, animal tissue culture has played a significant role in advancing our understanding of biological processes and has revolutionized various fields of research and practical applications. This article has provided a comprehensive overview of the history, fundamentals, roles, and requirements of animal tissue culture. By delving into the origins of tissue culture and highlighting its key techniques and applications, we have gained a deeper appreciation for the immense impact it has had on scientific discovery and medical advancements. As we continue to uncover new possibilities in the realm of

animal tissue culture, it is evident that this field will remain a cornerstone of modern biology, offering exciting avenues for further exploration and innovation. By grasping the fundamentals outlined in this article, readers can develop a solid foundation for their journey into the captivating world of animal tissue culture.

Also read :

- **[DOWNLOAD] Molecular Biology of the Cell The Problems Book 6th edition by John Wilson Tim Hunt**
- **Download Top 3 Genetics Books**