Chapter 1
Overview to Animal Cell Culture

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1.1 Introduction

The technique of taking cells from an animal or plant and growing them in an artificially controlled environment is known as cell culture. In many areas of the life sciences, cell culture has become a necessary tool. It lays the groundwork for researching cell proliferation, differentiation and product creation under tightly regulated settings. Cell culture has also allowed scientists to map nearly the entire human genome and examine the intracellular and intercellular signalling mechanisms that control gene expression.

From its origins in developmental biology and pathology, this discipline has evolved into a tool for molecular geneticists, immunologists, surgeons, bioengineers and pharmaceutical manufacturers, while remaining a critical tool for cell biologists, whose input is critical for the technology's continued development. As the new potential for genetic manipulation, whole animal cloning and tissue transplantation emerge, ethical as well as technical problems about gene therapy and tissue replacement are becoming increasingly important.

Despite major advancements in animal cell and tissue culture from the late 1800s, progress in animal tissue culture stopped until the early 1950s due to the lack of a viable cell line. The successful proliferation of cells generated from Mrs Henrietta Lacks' cervical cancer was demonstrated for the first time in the early 1950s. Mrs Henrietta Lacks' cells in culture revolutionised medical and biological research, enabling for major cellular, molecular and therapeutic breakthroughs, including the development of the first efficient polio vaccine (Rodríguez-Hernández et al., 2014; Del Carpio 2014). This culture is now known as HeLa, and there are

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more than 60,000 research publications, till 2017, on this cell line. Additionally, different scientific studies using HeLa cells have been engaged in multiple Nobel Prize-winning breakthroughs (Del Carpio 2014; Masters Masters, 2002; Schwarz et al., 1985). For the scientific study, animal cell culture is an important technique. Cell culture technology's usefulness in biological science has long been recognised. The isolation of cells from a tissue before creating a culture in an appropriate artificial environment is the first step in animal cell culture. Disaggregation employing enzymatic or mechanical procedures can be used to separate the cells from the tissues. The isolated cells are normally produced from an in vivo environment, but they can also be derived from an existing cell line or cell strain. Cell culture technologies have become an important tool for evaluating the efficacy and toxicity of new medications, vaccines and biopharmaceuticals, as well as for assisted reproductive technology.

Animal cell culture is one of the most essential and versatile procedures used in today's research. The following factors can be investigated using animal cell culture as a model system:

- Drug development and screening
- Carcinogenesis and mutagenesis
- Cellular physiology and biochemistry in their natural state
- Drugs and hazardous chemicals' potential effects on cells

Furthermore, it allows for dependable and repeatable results, making it an important model system in cellular and molecular biology.

Mammalian cell culture necessitates a favourable growing environment. Nutritional and physicochemical requirements are separated in environmental settings. A substrate or medium that offers support and necessary elements such as amino acids, carbohydrates, vitamins, minerals, growth factors, hormones and gases are all required (O_2, CO_2). All of these variables influence physical and chemical variables like pH, osmotic pressure and temperature. The majority of cells in animal tissue culture are anchorage-dependent and require solid or semisolid support in the form of a substrate (adherent or monolayer culture), whereas others can be cultivated directly in the culture media (suspension culture). Under controlled laboratory circumstances, animal, plant and microbial cells are always cultivated in a specified culture media. Microorganisms are more sophisticated than animal cells. It is challenging to estimate the optimal nutritional requirements of animal cells produced in vitro due to their genetic complexity. In comparison to microorganisms, animal cells require more nutrients, and they normally only thrive when connected to properly coated surfaces. Despite these obstacles, various types of animal cells, both undifferentiated and differentiated, can be successfully grown.

Tissue culture is the process of maintaining and propagating cells in vitro under ideal conditions. Animal tissue culture is the process of cultivating animal cells, tissue or organs in a controlled artificial environment. Animal tissue culture was first recognised as an important technique during the creation of the polio vaccine, which used primary monkey kidney cells (the polio vaccine was the first commercial product generated using mammalian cell cultures). Though these primary monkey

kidney cells were linked to a number of drawbacks (Van Wezel et al., 1978; Stones 1976; Beale 1981; Van Steenis et al., 1980), including:

1. The possibility of contamination by unknown substances (risk of contamination by various monkey viruses is high).
2. The majority of cells require anchoring and can only be cultivated effectively when attached to a solid or semisolid substrate (obligatorily adherent cell growth).
3. For virus generation, the cells are not well described.
4. Donor animals are in short supply because they are on the point of extinction.

Gay discovered that human tumour cells can produce continuous cell lines in 1951. As previously mentioned, the cell line regarded to be the first human continuous cell line was obtained from a cancer patient, Henrietta Lacks, and HeLa cells are still widely utilised. The most widely used resource in modern laboratories is continuous cell lines and are produced from human malignancies. Aside from advancements in cell culture, several media have been investigated, most of which are based on specific cell nutritional requirements, such as serum-free media, beginning with Ham's fully defined medium in 1965. Later on, hormones and growth factors were added to serum-free media in the 1970s to improve their performance. Thousands of cell lines are currently available, and a variety of media are available for their formation and maintenance.

1.2 Types of Cell Cultures

Animal tissue culture can be classified into two types; cultures that allow cell–cell interactions and stimulate communication or signalling between cells and cultures that do not allow cell–cell interactions and do not encourage communication or signalling between cells.

The first category is consisting of three different types of culture systems: organ cultures, histotypic cultures, and organotypic cultures are the three types of culture systems in the first category. On the other hand, cultures in monolayers or suspensions fall under the second type. Histotypic culture is the cultivating of cells for their re-aggregation to generate tissue-like structure, whereas organ culture is the culture of native tissue that retains most of the in vivo histological properties (Freshney 2005). Individual cell lineages are produced from an organ and then cultivated independently in a 3D matrix to explore interactions and signalling between homologous cells in histotypic cultures. Organ cultures are in vitro cultures of complete embryonic organs or tiny tissue pieces that retain their tissue architecture, i.e. the typical distribution of various cell types in the given organ (Edmondson et al., 2014).Cells from various origins are mixed together in specified proportions and spatial relationships in an organotypic culture to reform a component of an organ, i.e. the recombination of distinct cell types to create a more defined tissue or organ (Edmondson et al., 2014).

1.2.1 Primary Cell Culture

This is the first culture (a freshly isolated cell culture) or culture obtained by enzymatic or mechanical means straight from animal or human tissue (Freshney, 1987). These cells are slow-growing and diverse and have all of the characteristics of the tissue from where they came. The major goal of this culture is to keep cells growing on an appropriate substrate, which can be in the form of glass or plastic containers, in a regulated environment. They have the same karyotype (number and appearance of chromosomes in the nucleus of a eukaryotic cell) as the original tissue because they were taken directly from it. Once subcultured, primary cell cultures can give rise to cell lines that can either perish after a few subcultures (known as finite cell lines) or continue to proliferate indefinitely (known as indefinite cell lines) (these are called continuous cell lines). Normal tissues usually produce finite cell lines, but malignant cells/tissues (which are frequently aneuploid) produce continuous cell lines. Nonetheless, there are also unusual examples of non-tumorigenic continuous cell lines generated from normal tissues, such as MDCK dog kidney, fibroblast 3T3 and others. Mutation is thought to play a role in the evolution of continuous cell lines from primary cultures, altering their characteristics in comparison to finite lines (Jedrzejczak-Silicka, 2017). The possibility of genotypic and phenotypic variation can be increased by serial subculturing of cell lines over time. In contrast to primary hepatocytes, bioinformatic investigations based on proteomic characteristics showed that the Hepa1–6 cell lines lacked mitochondria, indicating a metabolic pathway rearrangements (Jedrzejczak-Silicka, 2017). With the introduction of advanced technology such as 3D culture, the utilisation of primary cells is becoming more common and yielding better results. There are two types of primary cells produced directly from human or animal tissue utilising enzymatic or mechanical procedures (Kim et al., 2020):

Adherent cells, also known as anchorage-dependent cells, are cells that require adhesion for proliferation. In other words, these cells have the ability to adhere to the culture vessel's surface. These cells are frequently obtained from organ tissues, such as the kidney, where the cells are stationary and entrenched in connective tissue.

Suspension cells, also known as anchorage independent cells, do not require any attachment or support in order to thrive. All suspension cells, such as white blood cells and lymphocytes, are extracted from the blood system and suspended in plasma. Cells grown from primary cultures have a short life span for a variety of reasons, i.e. they cannot be kept permanently. The exhaustion of the substrate and nutrients caused by a rise in cell populations in a primary culture can affect cellular activity and lead to the buildup of large amounts of hazardous metabolites in the culture. This could eventually lead to cell growth suppression. When a secondary culture or subculture must be produced to ensure continuing cell development, this stage is known as the confluence stage (contact inhibition).

1.2.2 Secondary Cell Culture

The first passaging of cells, a changeover to a different type of culture system, and the first culture derived from a primary culture are all examples of this (Segeritz & Vallier, 2017). This is commonly done after cells in adherent cultures have used up all of the available substrate or when cells in suspension cultures have exceeded the medium's capacity to sustain further growth and cell proliferation has slowed or stopped entirely. The primary culture must be subcultured in order to maintain appropriate cell density for continuing growth and to induce further proliferation. Secondary cell culture is the term for this procedure.

1.2.3 Cell Line and Cell Strain

A cell line is formed when a primary culture is subcultured or passaged. A continuous cell line is one in which cells continue to develop indefinitely throughout repeated subculturing, whereas finite cell lines incur cell death after several subcultures.

A cell line is a permanently formed cell culture that will multiply indefinitely if a suitable fresh media is continuously provided, whereas cell strains have been acclimated to culture but have a finite division capacity, unlike cell lines (Geraghty et al., 2014). A cell strain can be derived from either a primary culture or a cell line. This is accomplished through the selection or cloning of certain cells with predetermined qualities or characteristics (e.g. specific function or karyotype). In conclusion, the primary culture is the first culture that emerges from the in vivo environment. To create cell lines, this primary culture can be subcultured multiple times. Cell lines are immortalised or transformed cells that have lost control over division as a result of mutations or genetic abnormalities, or as a result of a primary cell being transfected with immortalising genes (Masters, 2002). Because most cell lines come from malignancies, they are tumorigenic (Verma et al., 2020). Cells produced from a primary cell line do not have this problem, although they are difficult to maintain. In most cases, primary cell cultures require a nutritional medium with a high concentration of various amino acids, minerals, and, on rare occasions, hormones or growth agents (McKeehan et al., 1990).

Primary cell cultures can be used effectively for two to four passes, after which the risk of contamination is higher than with cell lines. Primary cell cultures, on the other hand, have their own set of benefits too. The most common advantage of using primary cell culture is mainly due to their higher similarity with the organ system, they are isolated from.

1.3 Ethical Considerations in Animal Tissue Culture

Animal tissue culture procedures frequently use animal or human tissues, necessitating the development of animal research safety and ethics guidelines, commonly known as medical ethics. Animal handling brings up a slew of concerns that aren't present when using animal tissue. To begin research or study a human sample in the form of foetal materials or biopsy samples, the approval of the patient or his or her relatives is required in addition to the consent of local ethics bodies (Festing & Wilkinson, 2007).

A donor consent document in the required format should accompany any samples taken from a human donor. When working with human tissue, keep the following points in mind (Geraghty et al., 2014):

- The patient's or relative's permission to use tissue for research.
- Ownership of specimens, specifically cell lines and derivatives, with the recipient agreeing not to trade or transfer the cell lines and derivatives.
- Genetic modification consent, especially in the case of cell lines.
- For the commercial usage of cell lines, a patent or intellectual property right is required.
- The guidelines should be updated to reflect the most recent advancements in animal tissue culture research.

These recommendations are designed to provide appropriate information to newcomers, as well as to those participating in training and instruction. As using these informations, they may be better aware of the cell culture concerns, and if needed, they can effectively deal with those concerns. The following are the key areas of attention in the guidelines:

- Cell line acquisition
- Cell line authentication
- Cell line identification
- Cell line cryopreservation
- Cell line development
- Cell line instability
- Legal and ethical considerations in the creation of cell lines from human and animal tissues
- Microbial infection of the cell line
- Cell line misidentification
- Equipment selection and maintenance
- Cell line transfer between laboratories

1.4 Common Nomenclatures in Animal Cell Culture

The following are definitions of terms commonly used in animal tissue culture, particularly in the context of cell lines:

Adherent Cells. Cells that have the ability to stick to the culture vessel's surface using the extracellular matrix.

Immortalisation: Immortality is a term used to describe the state of being immortal. Obtaining a state of cell culture in which cells continue to proliferate.

Attachment efficiency: Within a certain period after inoculation, the fraction of cells that really cling to the surface of the culture vessel.

Passaging. Cell transfer is the process of moving cells from one culture vessel to another. Subculturing is a more descriptive name for the process of subdividing cells before transferring them to several cell culture containers.

A passage number indicates the number of times a cell line has been subcultured. When adherent cell cultures reach confluence (when they completely cover the surface of the cell culture tube), some will cease proliferating, and others will die if they are kept in this state for longer periods of time. As a result, adherent cell cultures necessitate recurrent passaging, which necessitates subculturing once the cells have reached confluence. In suspension cultures, where suspended cells utilise their culture media quickly, regular passaging is essential, especially when the cell density gets very high.

While culture maintenance necessitates frequent passaging, the process is more painful for adhering cells since they must be trypsinised. As a result, it is not recommended to passage adherent cell cultures more than once every 48 h.

Split ratio: The divisor of a cell culture's dilution ratio.

Generations number: The number of times a cell population has doubled in size. It's worth noting that passing and generation number are not synonymous.

Population doubling time: This value indicates how many times the cell population has doubled since isolation.

Passage number: The number of subcultures that culture has undergone.

Subculture: It is the process of transferring cells from one culture to another in order to start a new one.

Proliferating cells are subdivided during this process, allowing for the formation of new cell lines. When adherent cell cultures reach the confluent stage (i.e. when they completely cover the surface of the cell culture vessel), they will cease growing and will almost surely die if left there for a long time. As a result, adherent cell cultures should be passaged on a regular basis, meaning that when cells reach confluence, a portion of the cells should be passaged or subcultured to a new cell culture vessel. However, because adherent cells must be trypsinised, it is not recommended to subculture adherent cells on a frequent basis (no more than once per 48 h). Suspension cultures with a high cell density, on the other hand, necessitate frequent passaging because they consume medium quickly. The standard cell growth curve

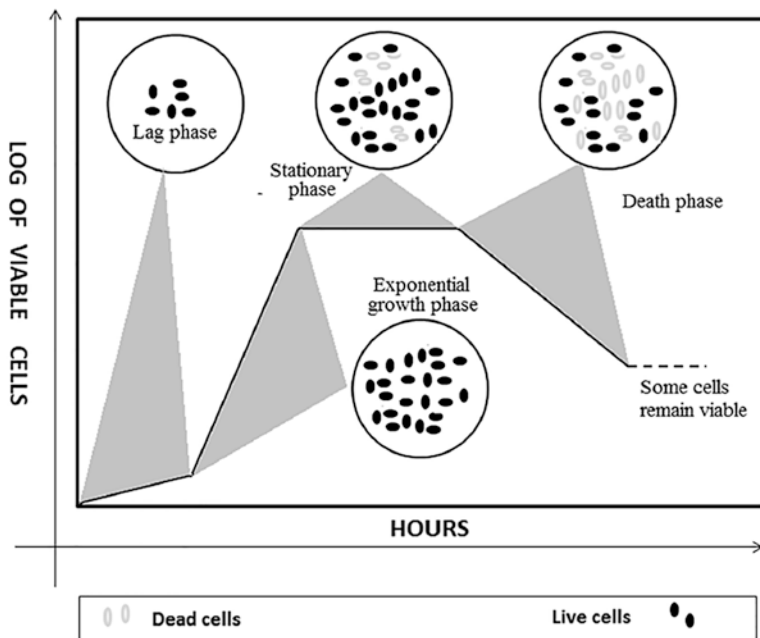

Fig. 1.1 Schematic representation for distribution of live and dead cells during different phases of cell growth in culture conditions

in culture is depicted in the Fig. 1.1, as mentioned- below. Because the cells have not yet accustomed to their new environment, there is less growth during the early lag phase. They grow exponentially as they begin to adapt to their surroundings, which is why this phase is known as the exponential or log phase. All cells are actively growing and consuming media at this moment. If the medium is not changed at this time, growth will come to a halt. As previously stated, the confluent phase occurs when the culture exceeds the medium's capacity. At this point, the culture must be broken down into subcultures. Current research practises necessitating the creation of good models, as good science cannot be accomplished with poor models. Several cell culture procedures, such as stem cell-derived human cells, co-cultures of different cell types, scaffolds and extracellular matrices, tissue architecture, perfusion platforms, organ-on-chip technologies, 3D culture and organ functionality, have been developed in the twenty-first century to overcome the drawbacks of traditional culture procedures and to be more scientifically rigorous (Fang & Eglen, 2017).

Organ-specific methodologies, more broad assessment of cell responses utilising high-content methods and the use of biomarker chemicals can all help to better the biological linkages between such models. A microphysiological model system can be created using these principles. One of the most notable benefits of this type of model system is that it produces results that are more similar to those seen in vivo; yet, managing many factors is a huge difficulty for the animal tissue culture industry.

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