ANIMAL BIOTECHNOLOGY

ANIMAL BIOTECHNOLOGY

Dr. S. Justin Raj, Dr. K. Jayala Jasmin, Dr. Bhagyasree S.R,
Dr. K.R. Beula Rani and Ms. Reshmi R. P

Authors: **Dr. S. Justin Raj, Dr. K. Jayala Jasmin, Dr. Bhagyasree S.R, Dr.K.R. Beula Rani and Ms. Reshmi R. P**
Coverdesign: **Dr. S. Justin Raj**
ISBN: 9789403906591
© **Dr. S. Justin Raj**

CONTENT

CHAPTER I

BASICS OF ANIMAL CELL CULTURE

Animal cell culture represents one of the most transformative pillars of modern biological sciences, evolving from a niche physiological curiosity into an indispensable industrial powerhouse. It is the complex process whereby cells are removed from a living organism and grown under strictly regulated artificial conditions, providing a window into the fundamental mechanisms of life without the ethical and physiological complexities of whole-animal experimentation. Over the last century, this field has transitioned from maintaining simple tissue fragments in primitive saline solutions to the sophisticated mass production of life-saving biopharmaceuticals, vaccines, and monoclonal antibodies. By bridging the gap between molecular biology and clinical medicine, animal cell culture has paved the way for breakthroughs in genetic engineering, toxicity testing, and the burgeoning field of regenerative medicine.

1. HISTORY AND DEVELOPMENT

1. The Early Foundations (1880 – 1910)

The roots of cell culture lie in "tissue culture," where researchers sought to keep organs or tissue fragments alive outside the body to study physiological processes.

- **1882 (Sydney Ringer):** Developed a balanced salt solution (Ringer's solution) containing sodium, potassium, and calcium chlorides, which allowed an isolated frog heart to keep beating outside the body. This proved that cells require specific inorganic ions for survival.

- **1885 (Wilhelm Roux):** Maintained the medullary plate of an embryonic chick in warm saline for several days, establishing the principle that tissue could survive independently of the organism.
- **1907 (Ross G. Harrison):** Often called the **"Father of Cell Culture,"** Harrison successfully monitored the growth of frog nerve fibers in a medium of clotted lymph. This was the first definitive proof that cells could grow and differentiate *in vitro*.

2. Methodological Refinement (1910 – 1940)

During this period, the focus shifted toward improving growth media and maintaining sterility, which was the biggest hurdle for early scientists.

- **Alexis Carrel (1912):** A French surgeon who introduced strict **aseptic techniques** to prevent bacterial contamination. He designed the "Carrel flask" (T-flask), which allowed for the exchange of air while keeping microbes out.
- **1913 (Steinhardt and Israeli):** Demonstrated the first medical application by growing the vaccinia virus in fragments of guinea pig corneal tissue.
- **1916 (Rous and Jones):** Introduced the use of the proteolytic enzyme **trypsin** to dissociate cells from tissue and from each other. This allowed for subculturing (passaging), enabling the creation of cell lines.

3. The Golden Era of Breakthroughs (1940 – 1970)

This era saw the transition from "natural media" (like lymph and plasma) to "defined media," as well as the birth of mass production.

- **Introduction of Antibiotics (1940s):** The discovery of penicillin and streptomycin revolutionized the field by drastically reducing the failure rate of cultures due to contamination.
- **The First Continuous Cell Line (1952):** George Gey established the **HeLa cell line**, derived from the cervical cancer of a patient named Henrietta Lacks. HeLa cells were "immortal," meaning they could divide indefinitely.
- **Vaccine Production (1954):** Enders, Weller, and Robbins won the Nobel Prize for growing the Polio virus in cultured monkey kidney cells, leading directly to the mass production of the Salk and Sabin polio vaccines.
- **Standardized Media (1955):** Harry Eagle identified the minimum nutrient requirements (amino acids, vitamins, salts, and glucose) for cell growth, leading to the creation of **Minimum Essential Medium (MEM)**.
- **The Hayflick Limit (1961):** Leonard Hayflick and Paul Moorhead showed that normal human cells have a finite lifespan and will eventually stop dividing (senescence), distinguishing them from immortal cancer cell lines.

4. Modern Biotechnology & Scalability (1970 – Present)

Modern developments focus on the industrial application of cell culture for therapeutics and regenerative medicine.

- **Hybridoma Technology (1975):** Köhler and Milstein fused antibody-producing B-cells with immortal myeloma cells to create hybridomas. This allowed for the mass production of **monoclonal antibodies**.
- **Serum-Free Media (1965/1970s):** Richard Ham and others developed media that did not require animal serum, reducing batch-to-batch variability and the risk of viral contamination.

- **Recombinant Proteins (1980s):** The use of Chinese Hamster Ovary (CHO) cells for producing complex human proteins (like Erythropoietin) transformed the pharmaceutical industry.
- **Current Trends:** Today, research focuses on **3D cell culture**, organ-on-a-chip technology, and the use of stem cells for tissue engineering.

Timeline of Important Discoveries

Year	Scientist	Contribution
1907	Ross Harrison	First successful frog nerve fiber growth *in vitro*.
1916	Rous & Jones	Use of trypsin for subculturing.
1952	George Gey	Establishment of the HeLa cell line.
1955	Harry Eagle	Development of chemically defined media (MEM).
1975	Köhler & Milstein	Hybridoma technology for monoclonal antibodies.

2. PLURIPOTENCY

Pluripotency refers to the unique biological potential of a stem cell to develop into all three primary germ layers of the embryo- **ectoderm, mesoderm,** and **endoderm.** These germ layers eventually give rise to all the tissues and organs in the human body, including the skin, nervous system, muscles, bones, blood, gastrointestinal tract, liver, and lungs. The hallmark of pluripotency is its broad differentiation capability, making pluripotent cells a vital resource for developmental biology, disease modeling, and regenerative medicine.

Pluripotent stem cells are primarily found in the inner cell mass (ICM) of the blastocyst-stage embryo, which forms around five days after fertilization. Two main types of pluripotent stem cells are extensively studied: **embryonic stem cells (ESCs)** and **induced pluripotent stem cells (iPSCs).** ESCs are naturally

pluripotent and are isolated directly from the ICM of a pre-implantation embryo. In contrast, iPSCs are artificially created by reprogramming adult somatic (body) cells into a pluripotent state. This is achieved by introducing a specific set of transcription factors—commonly **Oct4, Sox2, Klf4,** and **c-Myc-** which reset the gene expression pattern of the adult cell to resemble that of an embryonic stem cell.

Characteristics of pluripotent stem cells

Pluripotent cells possess several defining features that distinguish them from other cell types:

- **Self-Renewal:** One of the most critical features of pluripotent cells is their ability to undergo numerous cycles of cell division while retaining their undifferentiated state. This allows them to be cultured and expanded in the laboratory over long periods without losing their pluripotent properties.
- **Differentiation Potential:** These cells can differentiate into virtually any cell type of the body under appropriate culture conditions, making them invaluable for studying lineage specification and organ development. However, they cannot form extraembryonic structures such as the placenta or yolk sac (a capacity reserved for totipotent cells).
- **Marker Expression:** Pluripotent cells express a specific set of molecular markers that indicate their undifferentiated and pluripotent status. These include surface antigens such as SSEA-3, SSEA-4, TRA-1-60**,** and TRA-1-81**,** as well as transcription factors like Oct4, Nanog**,** and Sox2**,** which are essential for maintaining the pluripotent state.

Applications of pluripotent stem cells

The ability of pluripotent cells to give rise to a wide array of cell types has enabled numerous scientific and clinical applications:

- **Disease Modeling:** Patient-derived iPSCs can be used to model genetic diseases *in vitro*. By differentiating these cells into the affected tissue type (e.g., neurons for neurodegenerative diseases), researchers can study disease progression, identify biomarkers, and test therapeutic strategies in a controlled lab setting.
- **Drug Testing and Development:** Pluripotent stem cells offer a renewable source of human cells that can be used to test the efficacy and toxicity of new drugs. This reduces reliance on animal models and helps predict human responses more accurately.
- **Regenerative Medicine:** One of the most promising areas of stem cell research is the development of cell-based therapies to repair or replace damaged tissues. For example, pluripotent cells can be differentiated into **cardiomyocytes** for treating heart disease or **dopaminergic neurons** for Parkinson's disease.
- **Developmental Biology Studies:** These cells provide a model to study early human development, gene regulation, and cell fate decisions. By manipulating culture conditions and genetic pathways, scientists can explore how different cell types and tissues arise during embryogenesis.

3. CELL CULTURE MEDIA

Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established.

There are two basic systems for growing cells in culture, as monolayers on an artificial substrate (i.e., adherent culture) or free-floating in the culture medium (suspension culture). The majority of the cells derived from vertebrates, with the exception of hematopoietic cell lines and a few others, are anchorage-dependent and have to be cultured on a suitable substrate that is specifically treated to allow cell adhesion and spreading (i.e., tissue culture–treated).

Culture media

The culture medium is the most important component of the culture environment, because it provides the necessary nutrients, growth factors, and hormones for cell growth, as well as regulating the pH and the osmotic pressure of the culture. Although initial cell culture experiments were performed using natural media obtained from tissue extracts and body fluids, the need for standardization and media quality, as well as increased demand, led to the development of chemically defined media. The three basic classes of media are basal media, reduced-serum media, and serum-free media, which differ in their requirement for supplementation with serum.

Classes of media

Animal culture media can be divided into six subsets based on the level of defined media.

- Serum-containing media (commonly 10-20% FBS)
- Reduced-serum media (commonly 1-5% FBS)
- Serum-free media (synonymous with Defined media)
- Protein-free media (no protein but contains undefined peptides from plant hydrolysates)
- Chemically-defined media (with only recombinant proteins and/or hormones)

- Protein-free, chemically defined media (contains only low molecular weight constituents, but can contain synthetic peptides/hormones)
- Peptide-free, protein-free chemically defined media (contains only low molecular weight constituents)

Basal media

The majority of cell lines grow well in basal media, which contain amino acids, vitamins, inorganic salts, and a carbon source such as glucose, but these basal media formulations must be further supplemented with serum.

Reduced-serum media

Another strategy to reduce the undesirable effects of serum in cell culture experiments is to use reduced-serum media. Reduced-serum media are basal media formulations enriched with nutrients and animal-derived factors, which reduce the amount of serum that is needed.

Serum-free media

Serum-free media (SFM) circumvents issues with using animal sera by replacing the serum with appropriate nutritional and hormonal formulations. Serum-free media formulations exist for many primary cultures and cell lines, including recombinant protein–producing lines of Chinese hamster ovary (CHO), various hybridoma cell lines, the insect lines Sf9 and Sf21 (Spodoptera frugiperda), and for cell lines that act as hosts for viral production, such as 293, VERO, MDCK, MDBK, and others. One of the major advantages of using serum-free media is the ability to make the medium selective for specific cell types by choosing the appropriate combination of growth factors. The table below lists the advantages and disadvantages of serum-free media.

Advantages

• More consistent performance

• Easier purification and downstream processing

• Increased productivity

• Precise evaluation of cellular functions

• Better control over physiological response

• Enhanced detection of cellular mediators

Disadvantages

Requirement for cell type– specific media formulations

Need for higher degree of reagent purity

Slower growth

Chemically defined medium

A chemically defined medium (also known as synthetic medium) is a growth medium suitable for the *in vitro* cell culture of human or animal cells in which all of the chemical components are known. Standard cell culture media commonly consist of a basal medium supplemented with animal serum (such as fetal bovine serum, FBS) as a source of nutrients and other ill-defined factors. The technical disadvantages to using serum include its undefined nature, batch-to-batch variability in composition, and the risk of contamination.

There is a clear distinction between serum-based media and chemically defined media. Serum-based media may contain undefined animal-derived products such as serum (purified from blood), hydrolysates, growth factors, hormones, carrier proteins, and attachment factors. These undefined animal-derived products will contain complex contaminants, such as the lipid content of albumin. In contrast, chemically defined media require that all of the components must be identified and have their exact concentrations known. Therefore, a chemically defined medium must be entirely free of animal-derived components and cannot contain either fetal bovine serum, bovine serum or human serum. To achieve this, chemically defined media is commonly supplemented with recombinant versions of albumin and growth factors, usually derived from rice or *E. coli*, or synthetic

chemical such as the polymer polyvinyl alcohol which can reproduce some of the functions of BSA/HSA.

The constituents of a chemically defined media include: a basal media (such as DMEM, F12, or RPMI 1640, containing amino acids, vitamins, inorganic salts, buffers, antioxidants and energy sources), which is supplemented with recombinant albumin, chemically defined lipids, recombinant insulin and/or zinc, recombinant transferrin or iron, selenium and an antioxidant thiol such as 2-mercaptoethanol or 1-thioglycerol. Chemically defined media that are designed for the cultivation of cells in suspension.

The advantages of chemically defined media

- Avoidance of batch to batch variation of bovine serum or albumin, which causes inconsistency in growth-promoting properties.
- Low protein content, which can hinder product purification.
- Elimination of the risk of contaminants- viruses, mycoplasma, prions from animal-derived products which may be transmitted to the end product used by humans, e.g., bovine spongiform encephalopathy (BSE) or mad cow disease.
- Elimination of factors that may interfere with hormones or growth factors when studying their interaction with cells.
- Removal of concerns regarding the limited availability of fetal bovine serum, with periods of world shortages.
- Reduced cost: fetal calf serum can account for up to 85% of the overall cost of the medium when calculated for large-scale cultures.
- There are increasing concerns about animal suffering inflicted during serum collection that add an ethical imperative to move away from the use of serum wherever possible.

Chemically defined media also allows researchers who are studying in the field of cell physiology (especially extracellular) and or molecule–cell interactions to eliminate any variables that may arise due to the effects of unknown components in the medium.

4. BALANCED SALT SOLUTIONS (BSS)

Balanced Salt Solutions (BSS) are specially formulated aqueous mixtures of inorganic salts and buffering agents that replicate the ionic composition of body fluids, especially the extracellular fluid (ECF). They are essential in the field of **cell biology, tissue culture, and biomedical research,** providing a controlled environment that maintains cellular integrity and function during various experimental or clinical procedures. Their formulation ensures that key physical and chemical parameters- such as **osmotic pressure, pH, and ion balance-**are maintained to support cell survival outside the body.

These solutions are especially useful in short-term handling of tissues and cells, washing and transporting biological samples, and providing a stable medium during procedures such as cell dissociation or microscopy. Popular formulations include Hanks' Balanced Salt Solution (HBSS)**,** Earle's Balanced Salt Solution (EBSS)**,** and Phosphate-Buffered Saline (PBS)**.** Each of these has variations tailored for specific experimental conditions such as CO_2 dependency or calcium concentration.

Functions of Balanced Salt Solutions

1. Osmotic Balance

BSS maintains an isotonic environment, closely matching the osmolarity of body fluids (~280–300 mOsm/kg). This prevents cells from swelling (lysis) in

hypotonic solutions or shrinking (crenation) in hypertonic ones. Maintaining osmotic balance is critical for the structural stability of cell membranes and the functioning of intracellular enzymes and organelles**.**

2. pH Stabilization

Cells are highly sensitive to changes in pH. BSS includes buffering agents like **sodium bicarbonate** or **phosphate buffers,** which help maintain a physiological pH around **7.2–7.4**. The choice of buffer depends on the CO_2 concentration in the incubator. For instance, **bicarbonate-based buffers** are suitable for systems using **5% CO_2,** while **HEPES** buffers (in modified solutions) are ideal for ambient air conditions without CO_2 control.

3. Supply of Essential Ions

BSS provides cells with critical ions that are required for enzyme activity**,** membrane potential maintenance**,** and signal transduction. These ions include:

- **Sodium (Na^+)**: Maintains osmotic pressure and participates in transport mechanisms.
- **Potassium (K^+)**: Essential for maintaining membrane potential and nerve impulses.
- **Calcium (Ca^{2+})**: Plays a key role in cell adhesion, signaling pathways, and muscle contraction.
- **Magnesium (Mg^{2+})**: Serves as a cofactor for many enzymatic reactions and stabilizes DNA/RNA structures.
- **Chloride (Cl^-)**: Maintains charge balance and contributes to osmolarity.