Mammalian Cell Culture: An Overview

Srirupa Mukherjee, Parth Malik, and Tapan Kumar Mukherjee

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S. Mukherjee
Department of Immunopathology, Research Block A, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India

P. Malik
School of Chemical Sciences, Central University of Gujarat, Gandhinagar, India

T. K. Mukherjee (✉)
Amity University, Noida, Uttar Pradesh, India

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Abstract

The mammalian body typically consists of trillions of cells. These cells are the basic structural and functional units of a mammalian body. Together, these cells form tissues, tissues form organs, and organs collectively build up systems, constructing a whole body. The basis of cell theory states that mammalian cells originate from the pre-existent mother cells by the simple process of cell division (mitosis/meiosis). Thus, cell culture not only aims toward understanding the basic steps necessary for physiological processes involved in regular homeostasis in the body but also understanding various pathophysiological or diseased conditions associated with cellular dysfunction and their regulation by synthetic drugs/toxins, etc. Technically, **mammalian cell culture** is defined as the ongoing process by which cells are isolated from a mammalian body (typically from a specific tissue or organ) and are grown in vitro with a constant supply of synthetic nutrients under controlled laboratory conditions of optimum pH, temperature, and humidity in a CO_2 incubator/bioreactor. Nowadays, **the term tissue culture is often used interchangeably with cell culture**. In modern times, cultured mammalian cells are widely used for the bulk production of antibodies, vaccines, cytokines, growth factors, hormones, and other proteins in demand. To understand mammalian cell culture, it is not only important to gain knowledge regarding the basic concept and the ardent contributions of various scientists towards the development of this unique technology but it also requires selecting and validating specific experimental conditions for cell culture.

Keywords

History of mammalian cell culture · Karyotyping and authenticity of mammalian cells · Selection and validation of mammalian cells · Cell cycle and cell division in eukaryotes · In vitro culture of mammalian cells · Cell synchronization

1 Introduction

Evolutionary mammals are the most developed living creatures on this planet earth. Humans or *Homo sapiens* are positioned at the top of this evolutionary hierarchy. A mammalian body consists of trillions of cells that form the structural and functional foundations. (A typical human body may contain 30–40 trillion cells). Various cells form the tissues; tissues combine to generate an organ, and organs form an organ

system, which ultimately gathers and arranges together to give rise to a complex mammalian body (Martini and Nath 2009; Alberts et al. 2013).

It has been the eternal curiosity of the human mind to understand the genesis of new cells in a mammalian body. It is the handy proposal of ***Rudolf Virchow***, who modified cell theory, previously postulated by ***Matthias Jacob Schleiden*** and ***Theodor Schwann***, with Virchow's hypothesis being, "all organisms are composed of one or more cells and all cells come from preexisting cells." This fundamental concept indicates that **cell division** is the only process through which any living creature (including mammals) can get new cells (Silver 1987; Mazzarello 1999; Bradshaw and Stahl 2015).

In a cell, DNA synthesizes DNA by the process of **replication**, DNA synthesizes RNA by the process of **transcription**, and RNA synthesizes protein by the process of **translation**. This replication, followed by transcription and subsequently translation, is known as the **Central Dogma of Molecular Biology**. For a mammalian cell to divide (proliferate), every component, including DNA, RNA, and proteins, must be exactly doubled by the patterned events of replication, transcription, and translation, respectively. All the somatic cells (those which are not involved in reproduction) within a particular body are genetically exactly similar. However, within a cell at a particular time, certain genes may remain **transcriptionally switched on (or active)**, contrary to others that remain inactivated. This switching on or switching off of genes (**transcriptional regulation**) in somatic cells differs from one to another cell type. Thus, **due to differential expression** patterns, **mRNAs** and **proteins** expressed by one somatic cell may be absent in another somatic cell. Changes in the protein expression extents are known to be responsible for s**tructural (phenotypic)** and **functional** variations from one to the other somatic cell type (Crick 1970; Weber et al. 2007).

In vitro culturing of cells, tissues, and organs are one of the technologies that aid in a better understanding of the physiology and pathophysiology of the mammalian body. **Mammalian cell culture** is defined as the process by which cells are isolated from a mammalian body (specific organ or tissue) and are grown in vitro under controlled laboratory conditions of maintained **pH, temperature, humidity, O_2/CO_2** tension, and **readymade nutrient supply** (through cell culture medium). **Tissue culture** is the collective process by which whole tissues are derived (dissected) from a mammalian organ and are grown in vitro under controlled environmental conditions, either on a semi-solid, solid (**agar**), or liquid (**nutrient broth**) culture medium, supplemented with nutrients and growth factors, similar to that of cell culture. **Nowadays, the terminologies of tissue culture and cell culture are often used interchangeably** (McLimans 1972; Jacoby and Pasten 1979; Harris 1993; Clynes 1998; Mather and Barnes 1998; Davis 2011; Wilmer 2013).

This chapter describes the fundamentals of the discovery of cell/tissue culture techniques in multicellular eukaryotes, i.e., mammals. The year-wise chronological summary of major cell and tissue culture discoveries is cited in this chapter. Towards the end, the chapter sheds light on the factors necessary for the selection and validation of desired cells for one or more specific purposes.

2 Concept of Cells, Tissues, and Organs

Mammals are multi-cellular animals having eukaryotic (**EU** means developed, **KARYON** means nucleus) cells. Anatomically and physiologically, a mammalian body comprises different systems that are subdivided into various organs. Organs are further subdivided into more than one type of tissue. A particular type of tissue is composed of multiple similar types of cells. A cell is recognized as a fundamental structural and functional unit of life.

Ahead is a brief description of mammalian body constituents.

2.1 Cells of a Mammalian Body

Cells are the structural and functional units of all living organisms, including mammals. The term cell was first introduced by ***Robert Hooke*** in **1665** while observing the section of a dead cork through a simple microscope discovered by ***Anton van Leeuwenhoek***. In comparison to prokaryotic cells (**PRO** means primitive, **KARYON** means nucleus), mammalian cells are very **large**, with an average size ranging from (10 to 100) μm in diameter. Red blood cells (RBCs), one of the smallest human cells, have a diameter of around 8 μm and mature human RBCs do not have a nucleus. In the human body, the **longest cell** is the nerve cell. The **largest cell** in the **human** body is the female ovum. Male sperm is a very small cell. Regarding **shape**, mammalian cells exhibit significant diversity. In general, the cells are elongated or spherical. Some cells are long and pointed on both ends. Such cells exhibit a spindle **shape** (e.g., smooth muscle cells). In some cases, the cells could be very long. Some may be **branched** like the neurons or the nerve cells. In humans, there are about **200 different** cells and within these cells, there are about **20 different** structures or organelles (Schierbeek 1959; Hooke 1665; Gest 2004).

2.2 Tissues of a Mammalian Body

The word **tissue** means a group of cells that have a similar structure and act together to perform a specific function. The word **tissue** originated from a form of an old French verb meaning ***to weave***. A particular type of tissue contains a similar kind of cells. For example, in epithelial tissue, only various cells are present. In all multi-cellular organisms, cells, matrices, fibers, and many other materials help to form a tissue (Bryant and Mostov 2008).

The following four types of tissues form various mammalian organs:

Epithelial tissue
Connective tissue
Muscular tissue
Nervous tissue

Here is a brief presentation of the various tissues:

2.2.1 Epithelial Tissue

Marie François Xavier Bichat discovered epithelial tissue between 1771 and 1802. He was a French anatomist and pathologist and was subsequently remembered as the ***Father of Histology***. The epithelial tissue is made up of epithelial cells. Based on the specific shape, epithelial cells could be divided into **squamous, columnar**, and **cuboidal**. **Epithelial cells** are present in the outer layer of various body structures, including skin, organs, cavities, blood vessels, etc. The cells in epithelial tissue are tightly packed together with a very little intercellular matrix. Epithelial layers are **avascular** but **innervated**. The primary function of the epithelial tissue is the **protection** of all structures on the outer surface on which they reside. Other major functions of epithelial tissues are **secretion**, **absorption**, **excretion, filtration, diffusion,** and **sensory reception (**Fig. 1 describes various kinds of epithelial cells present in the human body).

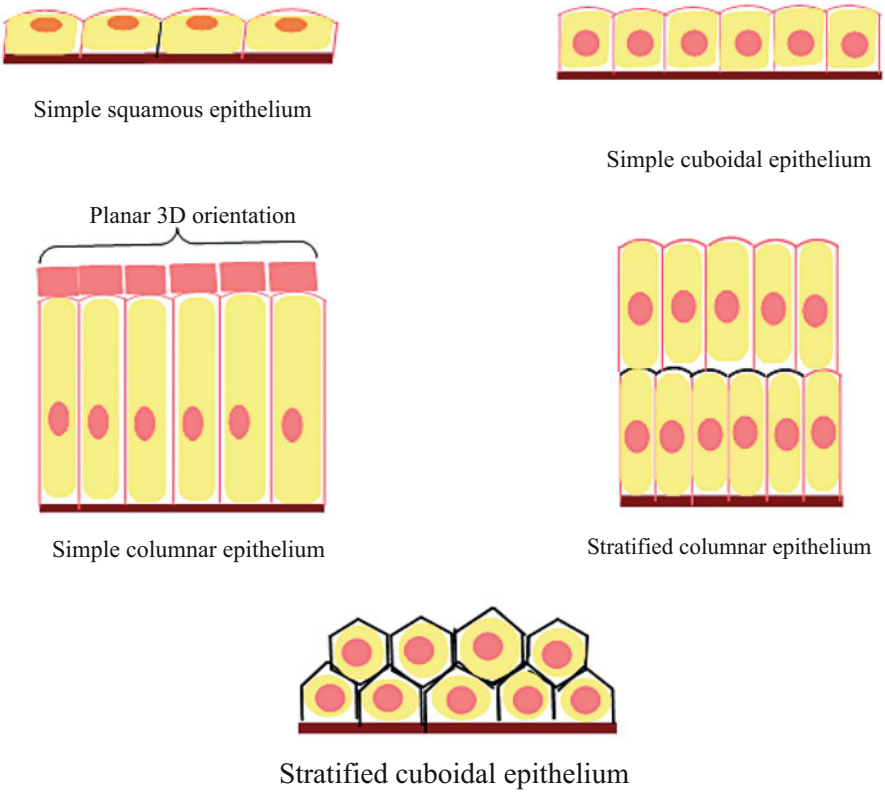


Fig. 1 Types of epithelial tissues lining body systems

2.2.2 Connective Tissue

Connective tissue is one of the four basic types of mammalian **tissue**. It develops from the **mesoderm of the embryo**. All connective tissue consists of three main components: **fibers, ground substance,** and **cells.** There are three types of fibers in the intercellular matrix of connective tissue: **collagen fibers, reticular fibers, and elastic fibers**. The most prevalent protein in the body is **collagen**, constituted of a triple helix structure. In ordinary connective tissue, the ground substance consists of water stabilized by glycosaminoglycans, proteoglycans, and glycoproteins. In bones, the ground substance includes minerals. In blood, the ground substance is **fluid** (plasma). The major cells present in connective tissue are fibroblasts, adipocytes, and various blood cells (e.g., RBC, WBCs, and platelets). **Blood is often called the liquid connective tissue**. As the name suggests, the basic function of connective tissue is to connect and communicate within cells, tissues, organs, and systems of a mammalian body. Other functions of connective tissues are binding and supporting, protecting, insulating, storing reserve fuel, and transporting substances within the body (Fig. 2 represents the fibrous connective tissue and blood, the liquid connective tissue).

2.2.3 Muscular Tissue

A type of tissue found in the mammalian body which functions by contracting, thereby exerting forces on different body parts. Muscle tissue consists of fibers of **muscle cells** connected in sheets and fibers. The muscle tissue is made up of **cardiac muscle** (present in the heart, semi-striated), **skeletal muscle** (attached to the skeleton, striated), and **smooth muscle** (present in the wall of the hollow visceral organ, non-striated). While cardiac and smooth muscles are involved in involuntary contraction, a skeletal muscle is involved in voluntary contraction. At the cellular level, each muscle cell has a complex of proteins containing **actin** and **myosin**. These proteins slide past one another when the contraction signal is received (**known as sliding filament theory, conceived by Hugh Huxley in 1953**). The filaments are connected to the ends of cells, and as they slide past one another, the cell contracts in length. A single cell can contract up to 70% in length, shortening the entire muscle in the process. Muscle tissue can be used to move bones, compress chambers, or

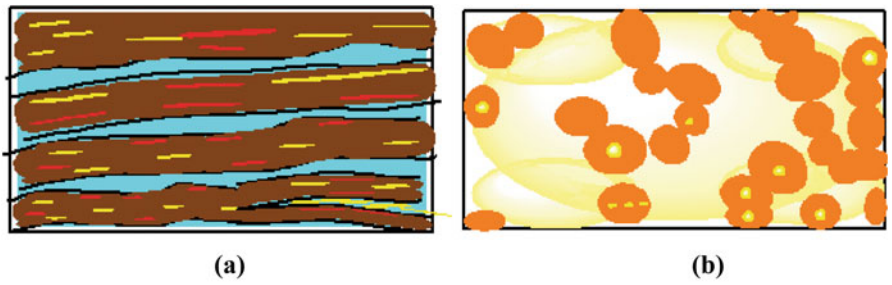


Fig. 2 Schematic representations of (**a**) fibrous connective tissue and (**b**) blood, the liquid connective tissue

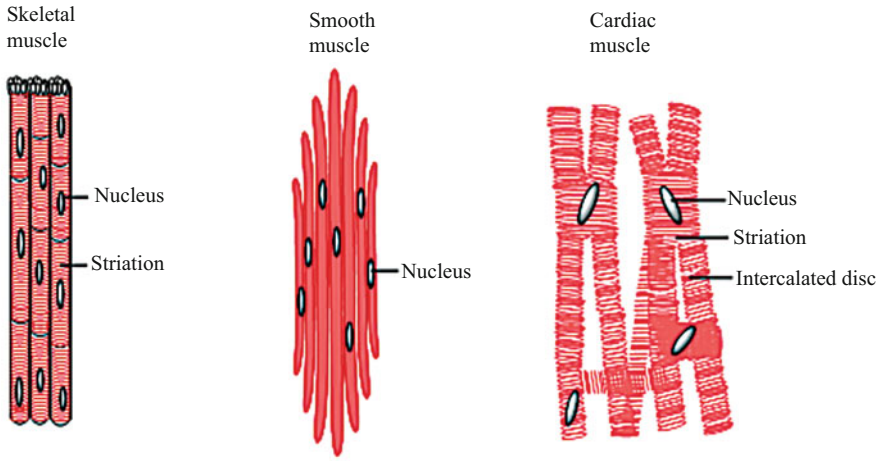


Fig. 3 Schematic description of muscular tissue diversities

squeeze various organs (Fig. 3. represents the three kinds of muscular tissues, i.e., skeletal muscle, smooth muscle, and cardiac muscle).

2.2.4 Nervous Tissue

Nervous tissue or neuronal tissue is a tissue that is made up of **neurons** or **nerve cells** and **neuroglial cells** (also called **supporting cells**). These cells are present in the central and peripheral nervous systems. A typical neuron consists of **Dendron and dendrites**, **the cell body (also called soma)**, and an **axon**. Dendrons and dendrites are responsible for responding to stimuli and receiving incoming signals towards the cell body. Dendrons and dendrites are motor neurons that are short and have a large surface area for receiving signals from other neurons. These convey incoming messages toward the cell body and are therefore called the receptive input regions. The cell body is like a factory for the neuron, producing all the proteins and containing specialized organelles such as the **nucleus, granules, and Nissl bodies.** The axon arises from the cone-shaped portion of the cell body called as **axon hillock**. Functionally, the axon is the conducting region of a neuron and is responsible for generating and transmitting impulses, typically away from the cell body. A single axon routes the nerve impulse from the cell body to another neuron or an effector organ. The axon can have many terminal branches, whereby there are chances of more than one cell getting stimulated each time, and nerve fibers. The axons are responsible for transmitting impulses over long distances from the cell body. The axon may be either **medullated** (covered with myelin sheath) or non-medullated (without myelin sheath). In the modulated axon, some regions of the myelin sheath are broken, which are known as **Nodes of Ranvier**, whereas in medullated nerve, ions only pass through the Nodes of Ranvier.

There are **six types of neuroglial cells**: four in the central nervous system **(CNS)** and two in the peripheral nervous system **(PNS)**. These glial cells are involved in

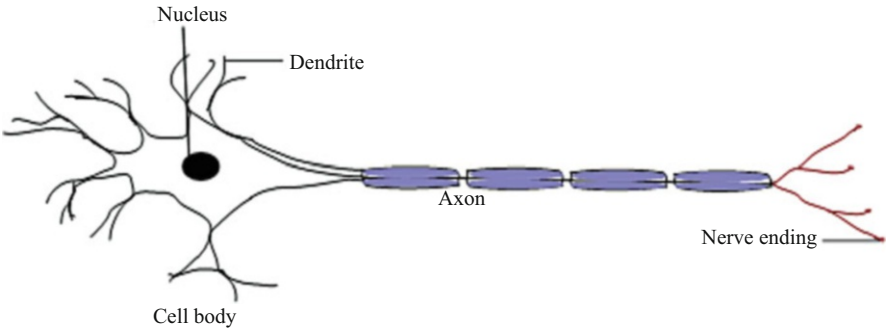


Fig. 4 Typical structure of a neuron, the functional unit of the nervous tissue

many specialized functions apart from the support of the neurons. Neuroglia in the CNS includes **astrocytes, microglial cells, ependymal cells, and oligodendrocytes**. In the PNS, **satellite cells and Schwann cells** are the two kinds of neuroglia. While neurons are highly specialized nerve cells that generate and conduct nerve impulses, neuroglias are supporting cells that provide physical support, remove debris, and provide electrical insulation. Thus, integration and communication are the two major functions of nervous tissue (Fig. 4 depicts a complete neuron).

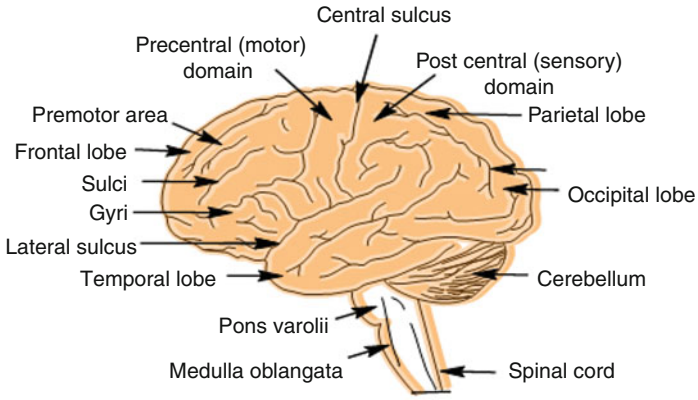
2.3 Organs of a Mammalian Body

Various tissues link together to form an **organ**.

The major vital organs of a mammalian body and their functions are as follows:

- The **Brain** controls thoughts, memory, and other organs.
- The **Heart** pumps blood throughout the body.
- The **Lungs** separate oxygen from the air and remove carbon dioxide from the blood.
- The **Stomach** helps to digest food.
- The **Intestines**, that aid in nutrient absorption from food.
- The **Liver** removes/breakdowns poisons (metabolites) from the blood.
- The **Kidneys**, filter blood and produce urine.
- The **Bladder** stores urine.
- The **Skin** guards the body and contains other organs.

The generalized structures of various vital human organs are depicted through different subsections of Fig. 5**.**



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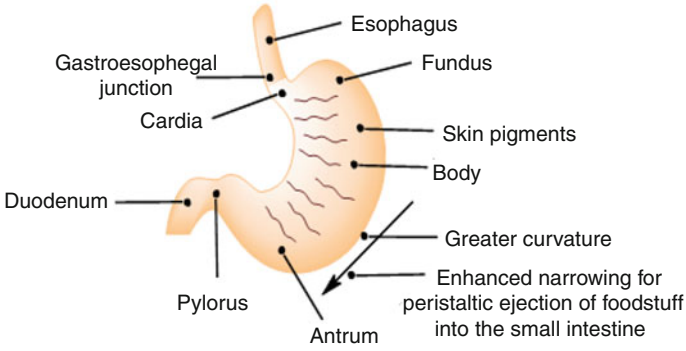

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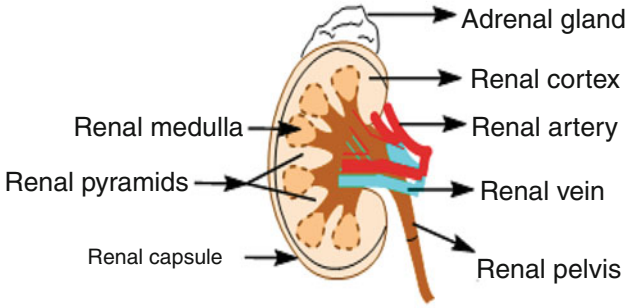
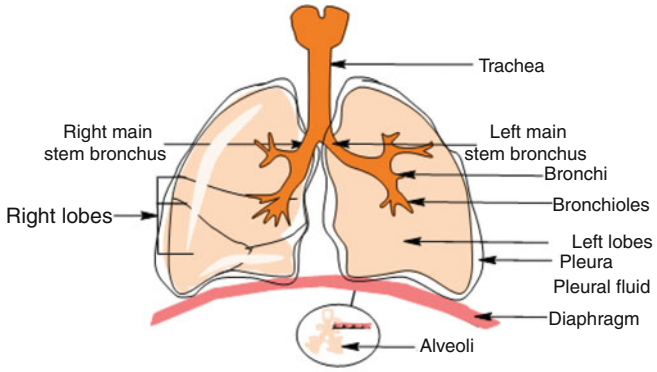


(c)

Fig. 5 Generalized structures of vital human organs, in a chronological manner as, (**a**) Brain, (**b**) Stomach, (**c**) Kidney (cross-sectional view), (**d**) Lungs (respiratory system), (**e**) intestines (digestive system), (**f**) liver, (**g**) gall bladder and (**h**) heart



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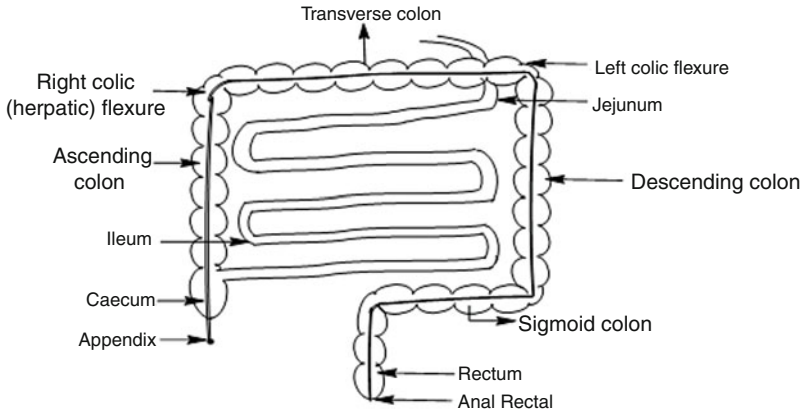

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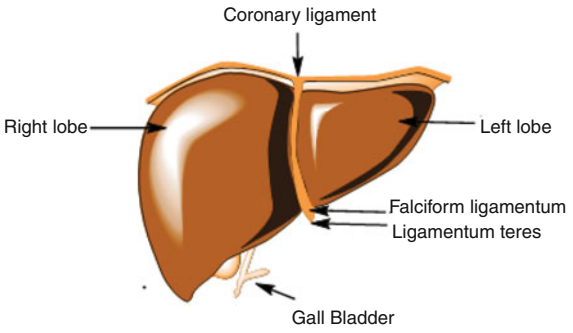
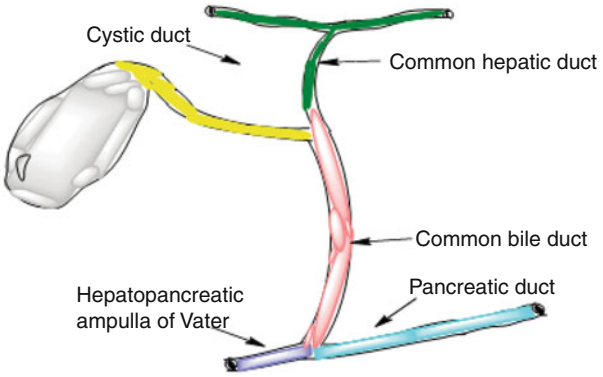
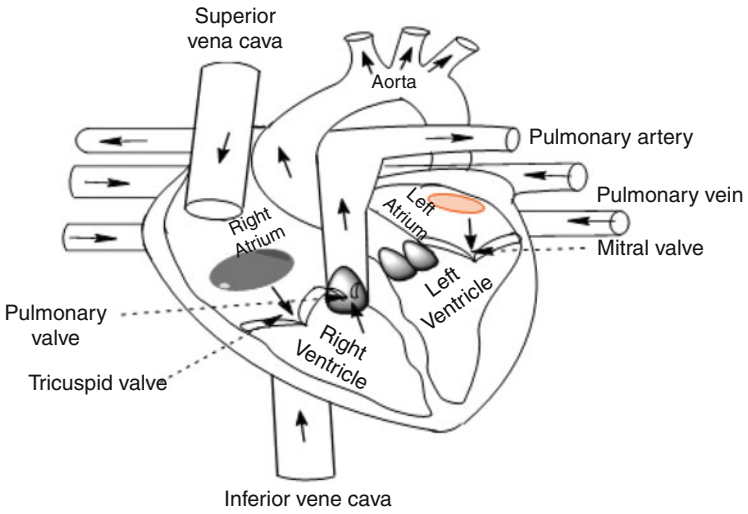


(f)

Fig. 5 (continued)



(g)



(h)

Fig. 5 (continued)

2.4 Organ Systems of a Mammalian Body

In a mammalian body, various organs are linked together to form a **system** (e.g., cardiovascular system consists of the heart, arteries, veins, and capillaries).

The major organ systems of the human body are as follows:

- **Nervous System**

It consists of cell body (Soma) and afferent and efferent neurons. It receives nerve signals, analyzes them, and sends a specific response to the effector organs.

- **Circulatory System**

It includes the heart, veins, arteries, and capillaries. Its function is to transport substances (e.g., nutrients, O_2, CO_2, metabolites, etc.) in the blood, throughout the body.

- **Respiratory System**

It includes the nose and the lungs. It takes in oxygen and removes carbon dioxide.

- **Digestive System**

It includes the mouth, esophagus, stomach, and small and large intestines. It breaks down food to absorb nutrients and water. It is also extended to the colon through which undigested food materials are passed out as stool.

- **Reproductive System**

It includes the vagina, uterus, fallopian tube, and ovary in women, and the penis, vas deference, and testes in men. Its function is to generate offspring via facilitating male and female gametes during reproduction.

- **Musculoskeletal System**

It includes bones and muscles. It supports the body and allows movement.

- **Immune System**

It includes bone marrow, thymus, spleen, lymph nodes, MALT, GALT, and immunological cells. It protects the body from infection and is described as a defense system of the human body.

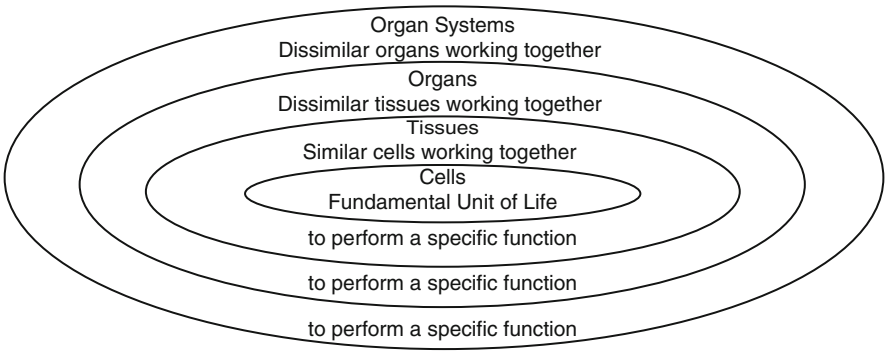


Fig. 6 Hierarchical make-up of the mammalian body, comprising of cells, tissues, organs, and organ systems. The organ systems collectively form a complete body, which works as a single unit

2.5 A Complete Mammalian Body

The physical substance of all the organ systems constitutes a complete mammalian body. Thus, a whole **body** is nothing but a superb arrangement of the various organ systems. Therefore, the formation of a whole-body system from cells, tissues, and organs, and finally organ systems, is the central theme of both ***mammalian anatomy and physiology.***

The process through which cells form a mammalian body is depicted in Fig. 6***, as follows:***

3 Basics of Cell Theory

Most cells are very small in dimension and are, therefore, visible only under a microscope. It is claimed that sometime around 1590, two Dutch spectacle makers, ***Zacharias Janssen*** and his father ***Hans***, started experimenting with lenses. They put several lenses in a tube and could able to see small molecules that cannot be seen with the naked eye. However, it was ***Antonie Philips van Leeuwenhoek*** whose microscopic observation of cells remains a breakthrough accomplishment in **Cell Biology**. A **microscope is considered** one **of the most essential instruments in a cell culture laboratory. Using this microscope, *Robert Hooke* first observed the cells**. Thus, the discovery of the microscope led to the discovery of cells, and sub-cellular structures, laying the foundation for understanding their fundamental behavior and characteristics. Cell theory comprises the gathering of all the meticulous research works in this field (Schierbeek 1959; Hooke 1665; Gest 2004; Masters 2008).

The theory was first developed in **1839** by ***Matthias Jakob Schleiden*** and ***Theodor Schwann*** and was later modified by ***Rudolf Virchow*** in **1858** (Alberts et al. 2013).

At present, the basic essence of cell theory revolves around the following important points:

- All organisms are composed of one or more cells, and **all cells come (originate) from pre-existing cells**.
- In an organism, all basic functions occur inside the cells, and all cells contain hereditary information for performing these basic functions.
- The hereditary information from chromosomes (DNA) and genes are transmitted to the next generation by the process of mother cell division into new daughter cells.

While cell theory described some important fundamental cell characteristics, it did not mention that the mammalian body comprising of not just one but two types of cells, **i.e.,** ***somatic and germ cells, and their division procedures may slightly differ.***

4 The Somatic and Germ Cells

A typical human body may have 200 different kinds of cells. Functionally, mammalian cells are divided into **somatic** and **germ (reproductive)** cells. Somatic cells form the whole body of a mammal except the reproductive organs. It indicates that every organ of a mammal is made up of somatic cells except those of the testes in males and ovary in females, which produce the reproductive or gamete or germ cells. The germ cells of testes are responsible for the production of **sperm in males**, while the germ cells in female (**Graafian follicle**) produce ovum. The somatic cells of humans contain a double number of chromosomes **(diploid, for humans it is 46)** compared to that of sperm or ovum, which contain half the number of chromosomes **(haploid, for humans it is 23)** to that of somatic cells. Different mammals (rat, mouse, human, etc.) may have dissimilar chromosome numbers in their cells. However, all somatic cells within the body of a particular species possess an exactly equal number and type of chromosomes. It indicates that every single human throughout the world contains exactly 46 chromosomes in each of their somatic cells and 23 lies in each reproductive cell. Of note, according to the **human genome project**, a typical human somatic cell may contain around 20,000 to 25,000 genes and may have an identical number of proteins. However, in the mammalian body, since genes may be present in several alternative forms, the actual number of proteins from a typical mammalian cell may be as high as 100,000. Interestingly, various genes may be functional (producing proteins) in different cells at the same instant.

Fundamentally, different phenotypes (morphology) and functional characteristics of various mammalian cells depend on the nature of **specific proteins expressed** by a particular mammalian cell. This further indicates that due to the **differential expression of various genes,** one type of somatic cell (e.g., neuron) may be morphologically, structurally, and functionally distinct from that of the other (e.g., smooth muscle cells). For example, while a neuronal cell looks like a tree and transmits a neuronal signal, a smooth muscle cell has a spindle shape and is involved in involuntary contraction. Every phenotypic characteristic is a reflection of the specific proteins produced by a particular cell type.

The fundamental rules of cellular characteristics are violated due to **spontaneous or induced genetic alterations (mutations) or epigenetic (beyond genetic) alterations, such as alteration in the pattern of DNA acetylation or methylation**, leading to the morphological and functional alterations of cells. This fundamental alteration gives rise to new types of cells, i.e., tumor or cancer cells. Of note, mutations in the germ cells are only inherited by the next generation, while somatic cell mutations only affect their implicit host and not the next generation.

Therefore, cells can be characterized based on their **karyotype (chromosomal characteristics)** and **morphology** (Puck and Fisher 1956; Nicholls et al. 2019).

5 Characterization of Cells: Karyotype and Morphological Analysis

Normally, mammalian cells are characterized by karyotype analysis and morphology.

5.1 Karyotype Analysis

The chromosomal characteristics of mammalian cells can be determined by karyotyping, a technique discovered by ***Levitsky***. Karyotyping determines the origin of a species and ascertains the extent of gross chromosomal changes in a cell line. Karyotypes describe the chromosome count of an organism and what these chromosomes look like under a light microscope. One must focus on their length, position of the centromere banding pattern, the differences between the sex chromosomes, and any other physical features. The preparation and study of karyotypes come under the umbrella of cytogenetics. The study of whole sets of chromosomes is sometimes known as **karyology**. The karyotype of cells is affected by the growth conditions, the pattern of cellular growth, and whether or not, the cells are preserved in the frozen state. Karyotypes can be used for many purposes, such as to study chromosomal aberrations, cellular functions, taxonomic relationships, and medicine and to gather information about past evolutionary events (Levitsky 1931; MacLeod and Drexler 2005).

5.2 Morphology Analysis

Most mammalian cells are morphologically sub-divided into three basic categories.

5.3 Epithelial

These cells are derived from all three germ layers, though the true epithelium is considered the **ectoderm of the growing embryo**. These cells are **polygonal and flattened** with more regular dimensions and grow as being attached to a substrate in discrete patches. Mostly, these cells could exist in **squamous** (flat or scale-like), **cuboidal** (height and width are the same), or **columnar** (cells are taller than wide) morphology.

Epithelial cells develop into sheets or tubes that separate an organism from its environment, allowing for the survival of multicellular organisms. Tissues encoding epithelial cells prevail throughout the body, including the epidermis, digestive, reproductive, and endocrine systems.

Epithelial cells could be **simple** (stomach, intestine, or kidney), **stratified** (epidermis, esophagus, tongue), or **pseudo stratified** (remaining attached to basement membrane). Simple and pseudo-stratified epithelia consist of a single cell layer resting on a basement membrane. In contrast, stratified epithelia consist of two or more layers, with only the dividing layer of cells remaining attached to the basement membrane. Crosstalk between epithelial cells permits the tissues to respond in a coordinated manner toward the growth, differentiation, and wound healing-related stimuli. In the transformed state, keratinocytes and other epithelial cells give rise to **carcinomas** (as and when there are genetic or epigenetic alterations).

In culture, epithelial cells grow while being adhered tightly to the substrate, resulting in **flattened morphology**. The cells assort into a contiguous monolayer due to contact inhibition, the process by which monolayers of cells stop dividing and migrate once they come in contact with each other. The intermediate filament protein **keratin** is also used as a **cellular marker** for epithelial cells grown in cell culture.

5.4 Fibroblastic

These cells are elongated and could be bi- or multipolar. They grow while being attached to a substrate. Fibroblasts are typically present in almost every tissue type, originating from **embryonic mesoderm**, evidenced by the presence of intermediate filamentous protein **vimentin**. This protein, along with actin and tubulin, forms the structural support of the cell. In vivo, fibroblasts are generally wider in the middle and tapered at the ends, while remaining embedded within the connective tissue. Fibroblasts secrete proteins that are important for extracellular matrix (**ECM**) formation, including **collagen**, **elastase**, **fibronectin**, **and laminin**. These are also known to play important roles in normal physiological processes, such as wound healing through the secretion of matrix proteins, growth factors, and cytokines. In the event of injury, tissue damage stimulates fibroblasts to differentiate into myofibroblasts, which contract to enclose a wound.

These exhibit contact inhibition to keep fibroblasts from growing into monolayers when in contact with neighboring cells. Fibroblasts are also well-known for their decisive roles in many diseased states. Overproduction of connective tissue can

change its normal morphology, resulting in a state known as **fibrosis.** Transformed fibroblasts can also give rise to a cancerous form, called **sarcoma**, comprising of tumors with a mesenchymal origin. In general, fibroblasts maintain their characteristic **fusiform morphology**, but in the G/M phase of the cell cycle, they may adopt **a rounded shape** in course of preparation for cell division (mitosis and cytokinesis).

5.5 Lymphoblastic

These are immature cells that on differentiation give rise to mature lymphocytes (**B and T cells**), natural killer (**NK**) cells, and natural killer type T cells (**NKT**). They are normally found in the bone marrow, are generally **round or spherical,** and are usually **grown in suspension** without surface attachment. Lymphoblast typically modifies naïve lymphocytes (immune-responsive cells formed on account of leukocyte differentiation) with an altered morphology. It is **matured** after the activation of lymphocytes by an antigen (from **Antigen Presenting Cells**) and is increased in volume by the nucleus and cytoplasmic growth, as well as new mRNA and protein synthesis. After this stage, a lymphocyte undergoes division two to four times per 24 h for (3 to 5) days. Lymphocytes give rise to **T (T denotes Thymus) and B (B denotes Bursa Fabricius in birds) cells**, responsible for **cellular** and **humoral immunity**, respectively. T lymphocytes further differentiate into **T-helper (Th), T-suppressor (Ts), and T-cytotoxic (Tc) cells**. Helper T cells (**TH1 and TH2**) stimulate antibody production and are released in B-cells. Non-stimulated T and B cells are very similar in appearance, even when imaged using a high-resolution electron microscope. Both these cells are only slightly larger than RBCs, with very little cytoplasm being observable owing to the nucleus occupying a major cell volume.

Besides, **Neuronal Cells** have distinct morphological characteristics. They exist in different shapes and sizes but are roughly divided into two basic morphological categories: **type I** with long axons used to move signals over long distances and **type II** without axons. A typical neuron projects cellular extensions with many branches from the cell body, which is referred to as a dendritic tree. These morphological characteristics are specific to their particular role in a host. These distinctions are quite evidently observed amidst the neuron development and functional coordination in vertebrates and invertebrates.

At the early stage of cell biology research, one of the important questions that cell biologists tried to resolve is, ***"Whether Mammalian Cells/Tissue/Organs Can Be Cultured In Vitro?"***

The following paragraphs illustrate a brief interface on the basic concept of mammalian cell division, followed by cell/tissue/organ culture and the important discoveries linked to this section.

6 Concept of Increasing the Number of Cells: The Process of Cell Division

The concept of increasing the number of cells,* i.e., *cell division, is represented by three small subsections. They are as ahead:

1. A Basic Concept of Cell Division in Eukaryotes
2. Cell Cycle and Its Importance in Cell Division
3. Cell Synchronization and Its Importance in Experimental Cell Culture Research

Here is a brief discussion about them:

6.1 A Basic Concept of Cell Division in Eukaryotes

Cell division is a normal physiological phenomenon through which one somatic cell divides into two by the process of **mitosis** and one reproductive or germ cell divides into four cells initially by the process of mitosis, which is followed by **meiosis**. In humans, while in mitosis the phenotype and genotype of the mother and the two daughter cells, both are exactly similar, i.e., they contain **46** chromosomes, every germ cell, i.e., all the four germ cells contain **23** chromosomes only. Since cell division increases the number of cells, it is also called **cell proliferation**. In mammals, cell division is required not only for the **embryogenesis of a fertilized zygote** and the **morphogenesis of a growing fetus to form a complete body** (Figs. 7 and 8) but also to **replenish or** renew the old dying cells and **to repair** the damaged tissues and organs.

The role of mitosis is to replicate the genetic material in an existing cell, which is known as the **"parent cell,"** and to distribute that genetic material into two new cells, known as **"daughter cells."** The main purpose of mitosis is to accomplish cell regeneration, cell replacement, and growth in living organisms**.** Mitosis is important because **it ensures that all new cells generated in a given organism have the same number of chromosomes and genetic information**. Mitosis occurs in four discrete, consistently consecutive phases: (1) **Prophase**, (2) **Metaphase**, (3) **Anaphase**, and (4) **Telophase** (Fig. 9**)**.

6.1.1 Prophase

The first step of mitosis is called prophase. This is when the genetic fibers within the cell's nucleus, known as chromatin, begin to condense and become tightly compacted together. Once the chromatin (a mixture of chromosomes and proteins) has been condensed into individual chromosomes, the genetically identical chromosomes come together to form an "X" shape, called **sister chromatids**, which join at the **kinetochore** through **centromeres**. Slowly, spindle fiber is formed through microtubule proteins. At the **late prophase** or **early metaphase**, the nuclear membrane breaks down, leading to the commencement of the next phase.

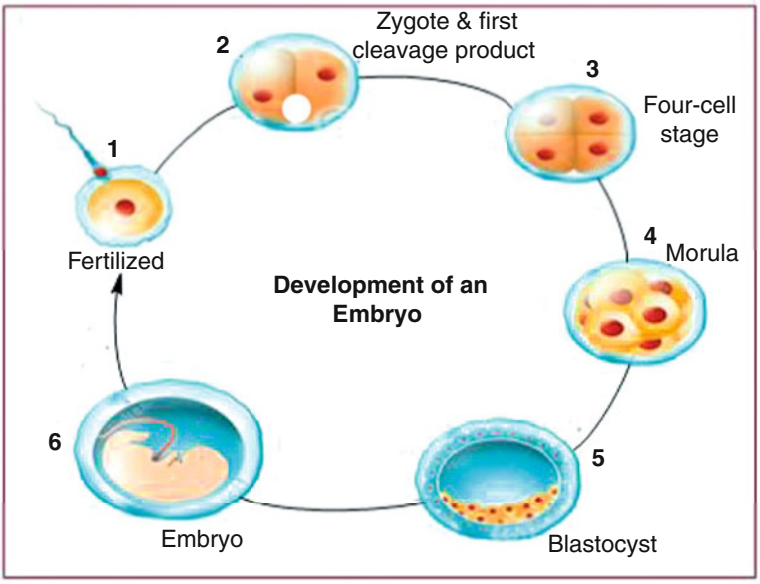


Fig. 7 Schematic stages involved in embryogenesis of a fertilized zygote. (Figure drawn by extracting inputs from https://www.shutterstock.com/image-vector/embryo-development-fertilization-zygote-morula-blastocyst-1441785692)

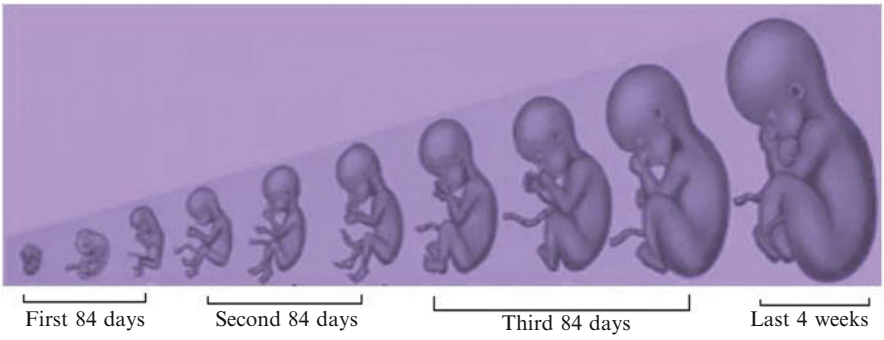


Fig. 8 Morphogenesis of a growing fetus to form a complete body. (Figure made after referring to https://www.babydestination.com/stage-wise-development-of-fetus)

6.1.2 Metaphase

Metaphase is the second step of mitosis. At the beginning of this phase (prometaphase), all the kinetochore microtubules get attached to the sister chromatids' centromeres. In the next step, the sister chromatids are equitably distributed across the metaphase plate. At this stage, the **metaphase** checkpoint screens the readiness of cells for the division which includes examining whether kinetochores are properly attached to the mitotic spindles and that the sister chromatids are **evenly distributed**

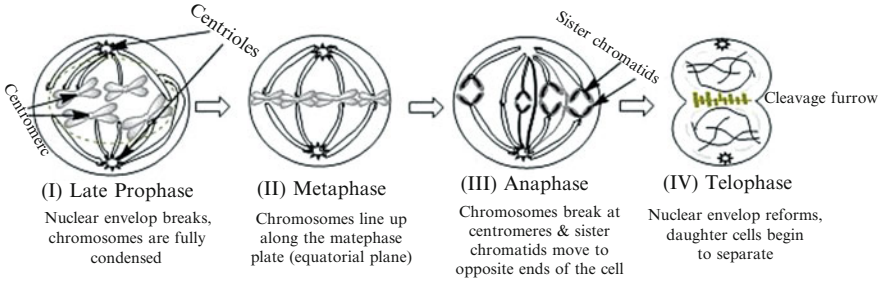


Fig. 9 Sequential stages of mitosis, responsible for maintaining a constant number of chromosomes and genetic information in all the new cells formed in an organism

and aligned across the **metaphase plate**. The positive results of the metaphase checkpoint allow the cells to move to the next phase of cell division.

6.1.3 Anaphase

Anaphase is the third phase of mitosis. It is in this phase that the two chromatids separate into two identical chromosomes and are subsequently **pulled toward the opposite ends of the cells** by the spindle fibers. This phase ensures that each chromosome receives identical copies of the parent cell's DNA. Since chromosomes are pulled at the two ends of cells, they exhibit **maximum condensation**.

6.1.4 Telophase

The **last phase** of mitosis is called telophase. This phase is characterized by the separation of telophase when the newly separated daughter chromosomes get their nuclear membranes with identical sets of chromosomes. This new nuclear envelope forms around the two sets of separated daughter chromosomes, **creating two separate nuclei inside the same cell**. Now that the two sets of daughter chromosomes are encased in a new nuclear envelope, they begin to spread out again. With the division of one nucleus into two identical nuclei, the completion of mitosis is inferred.

6.1.5 Cytokinesis

Cytokinesis is the actual division of the cell membrane, including the cytoplasm, into two discrete cells. For completing the process of cell division, cytokinesis is highly essential. To begin cytokinesis, a contractile ring (constituting protein filaments) that forms down the middle of the cell starts shrinking, pulling the cell's outer plasma membrane inwards. Eventually, the contractile ring shrinks so much that the plasma membrane pinches off and the separated nuclei can form into their cells. The end of cytokinesis signifies the termination of the M-phase of the cell cycle, of which mitosis is also a part. At the end of cytokinesis, the division phase of the cell cycle has formally ended (Alberts et al. 2002; Golitsin and Krylov 2010).

NB: While in mitosis (the division of somatic cells), one mother cell (2N) divides into two daughter cells (2N each), in meiosis (a division of germ or reproductive

cells,* e.g., *sperm and ovum), one mother cell (2N) divides into four daughter cells (each containing N number of chromosomes). This decrease in the number of chromosomes helps to maintain the number of chromosomes in offspring) as and when germ cells are fertilized (N from sperm + N from ovum = 2N).

6.2 Cell Cycle and Its Importance in Cell Division

Immediately after mitosis, when a mother cell is divided into two daughter cells, both these cells undergo a resting phase ($\mathbf{G_o}$). A G_o phase indicates that a cell is not doing anything related to cell division, including the preparation for cell division. A complex regulatory mechanism controls if and when these cells will transit from the Go phase to the preparation for cell division by the **G1 phase** (synthesis of every protein for DNA replication), **S phase** (DNA replication phase), and **G2 phase** (checking whether every material necessary for cell division is synthesized in adequate and just quantity), and finally cell division at **M phase**. This cycle of events (**Go-G1-S-G2-M**) is repeated again and again in the life cycle of a **dividing cell** (neuronal cells, mature erythrocytes, etc. are non-dividing cells and remain in the Go phase for their entire life span) as long as a cell undergoes division. While a normal cell shows a limited number of divisions (**Heyflick effect**), a cancer cell may have a very short or no Go phase and is capable of unlimited division, provided proper nutrients are supplied (Hayflick and Moorhead 1961; Rubin 1997; Wright and Shay 2002; Jafri et al. 2016).

Two classes of proteins control the regulation of the cell cycle. They are called **cyclins** and cyclin-dependent kinases (**CDKs**), a type of serine/threonine kinases. Cyclins are regulatory subunits, and CDKs are catalytic subunits of the same enzyme. Every phase of the cell cycle is regulated by specific cyclins and CDKs. For example, in mammalian cells, **cyclin D** (D1, D2, D3) regulates the G1 phase, while **cyclin E** (E1, E2) is involved in the (G1-S) phase transition, **cyclin A** (A1, A2) is predominantly involved in the S phase entry, and **cyclins B** (B1, B2, B3) are predominantly involved in G2-M transition. Each cyclin needs to bind with a specific CDK for its action. For example, in mammals, cyclin D binds with CDK4 and CDK6, cyclin E binds with CDK2, cyclin A also binds with CDK2, and cyclin B binds with CDK1. While a particular cyclin level rises during activation of a particular cell cycle phase and its level generally goes down after their activity (**hence called cyclins because** of their **cyclic appearance and disappearance**), the CDKs are maintained in their steady-state level at various cell cycle phases.

For example in the resting phase (Go), the cyclin D level of the cells is very low. As the cells enter the G1 phase, the cyclin D level increases, binding to CDK4 and CDK6 and activates the G1 phase by preparing all the proteins necessary for DNA synthesis (replication). Once the G1 phase is over, the cyclin D level again decreases due to their degradation. The cyclin D level once again increases in the next phase of the cell cycle when there is a need to replicate the DNA. That is why the cyclin name was given **(cyclic appearance and disappearance in various cell cycle phases)**.

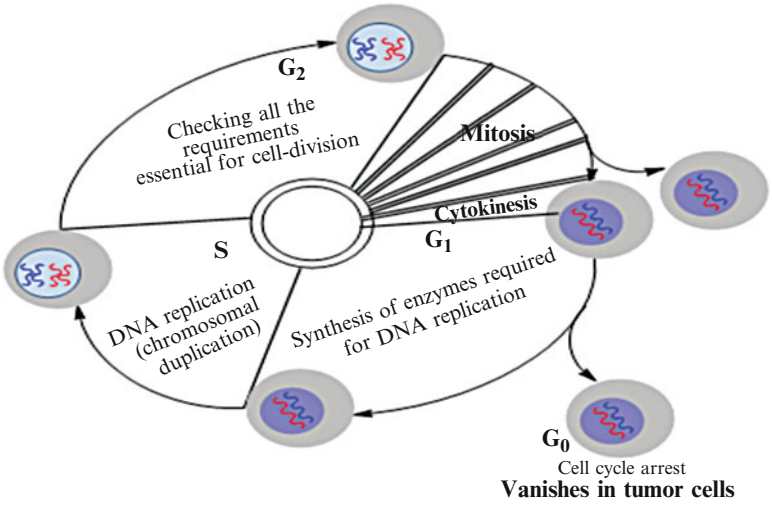


Fig. 10 Schematic description of cell-cycle stages

For experimental purposes, one can identify and subdivide a particular population of cells belonging to the G1 or S or G2 or M phase. An instrument called **a cell sorter or flow cytometer** effectively sorts out the cells based on the specific proteins that are involved in the progression of a particular cell cycle phase. In flow cytometry, antibodies conjugated with various fluorescent molecules such as **FITC, FC, or Avidin-biotin** are utilized to bind cell cycle phase-specific proteins. A flow cytometer is not only useful to cell biologists and immunologists but also to cancer biologists. As mentioned previously, cancer cells are marked by a continuous cell cycle progression without any Go phase (Morgan 2007). Figure 10 represents various cell cycle phases and their functions.

6.3 Cell Synchronization and Its Importance in Experimental Cell Culture Research

To observe the efficacy of a drug/toxin/medicinal preparation, etc. on the experimental cultured cells, **synchronization** of the cells is highly essential. Synchronization is the state in which all the cells are in a desired phase of the cell cycle. The most desired phase to stop the cell cycle is G1, since the **G1-S phase transition** is responsible for effectively initiating the cell cycle. Henceforth, the most important transition point is the **G2-M phase transition**, where cells are checked for the necessary preparations needed for cell division in adequate quantity before entering the process of cell division (mitosis/meiosis) (Harper 2005). The easiest way to stop the cells at the G1 phase of the cell cycle is **serum starvation** (withdrawal of FBS/**FCS from the cell culture medium**).

Some of the important drugs/molecules that are effective in various phases of the cell cycle are as follows:

6.3.1 Synchronization of the Cells in the G1 Phase of the Cell Cycle by Serum Starvation

Technically, at the Go phase of the cell cycle, cells are not doing anything related to cell division. Just before the commencement of the G1 phase, cells must ensure that negative inhibitory signals mediated by the tumor suppressor proteins (such as P^{53}, P^{Rb}, P^{27}, P^{21}, PTEN, etc.) are secreted at the lowest (basal) extent while the positive stimulatory signals mediated by various materials (e.g., growth factors, hormones, amino acids, etc.) are at their highest level. For in vitro mammalian cell culture, fetal calf serum (FCS) or fetal bovine serum (FBS) are added to the cell culture medium, to supply nutrients, growth factors, hormones, vitamins, minerals, etc. These molecules are necessary for synthesizing all the proteins/enzymes for DNA replication. Thus, starving cells of serum can prevent them from division. While serum starvation is the cheapest way to synchronize the cells in the G1 phase of the cell cycle, some cell lines may not be sensitive to serum starvation.

The process of serum starvation is as follows:

- Take freshly cultured cell culture containers having 60–80% confluency of exhibiting cells.
- Remove the serum-containing medium from the cell culture containers.
- Wash the cell culture containers either with Phosphate Buffer Saline (**PBS**), Hanks Balanced Salt Solution (**HBSS**), or cell culture medium without any additives, including serum.
- Add a complete serum-free medium to the culture containers.
- Incubate the cells in a CO_2 incubator for 24 to 72 h.
- Check for synchronization in the G1 phase using DNA dyes in flow cytometry.

NB: Many cells cannot tolerate the complete withdrawal of serum from the culture medium. For example, the endothelial cells perish within 18–24 h of complete serum starvation. Also, sudden/steep removal of serum from the cell culture medium may have drastic effects contrary to the step-wise gradual serum starvation.

6.3.2 A High Level of Thymidine Blocks Cells in the G1 Phase and Causes Cell Synchronization

While thymine (Dexythymidine) is one of the nucleotides needed for DNA synthesis (DNA replication), a high concentration of thymine blocks DNA synthesis. Based on this basic principle, a high thymine concentration is used to synchronize cells at the G1-S phase transition. Of note, while the G1 phase is necessary for the synthesis of all the proteins/enzymes necessary for replication, the S phase is responsible for the actual DNA synthesis.

The process of a double thymidine block is as follows:

- Seed and culture the mammalian cells in vitro.
- Allow them to grow till the mid- to early-log phase (40–50% confluency).

- Add 2 mM thymine to the culture medium and incubate overnight (~ 10–12 h).
- Wash the cells to remove excess thymine present in the medium.
- Incubate the cells with **deoxycytidine** (~ 9 h) to release them from the block.
- In simple terms, deoxycytidine restores the imbalances in nucleotide pools that occur after exposure to excess thymidine.
- Repeat the thymidine incubation.
- Release again using deoxycytidine.
- Check the blocking at the G1-S phase transition using flow cytometry.

6.3.3 Inhibition of Cyclin-Dependent Kinases Arrests the Cells at the G1 Phase

- As described in the previous paragraphs that cyclins and CDKs are two separate classes of proteins that collectively regulate various cell cycle phases. Each phase of the cell cycle is regulated by different classes of cyclins and CDKs. Collectively, while specific cyclins are involved in phase implicit cell cycle regulations, CDKs are involved in the catalytic activities.
- Based on this principle, several CDK inhibitors have been recognized that arrest the cell cycle via inhibiting the actions of various CDKs.
- For example, cyclin D (D1, D2, and D3) and CDK4/CDK6 are involved in the activation of the G1 phase of the cell cycle.
- CDK4/6 inhibitors are very effective for arresting mammalian cells in the G1 phase.
- Large pharmaceutical companies such as Pfizer, Novartis, Eli Lilly, etc. have developed several CDK inhibitors as anticancer drugs.
- Some examples of CDK inhibitors are **palbociclib** (Pfizer), **ribociclib** (Novartis), and **abemaciclib** (Eli Lilly).
- Proper optimization is needed for these CDK inhibitors' actions since some of these may have off-target effects at various concentrations (Pardee 1989).

NB: ***A large number of cells cannot tolerate serum starvation and eventually die. Similarly, thymidine block is a laborious and time-consuming technique. On the other hand, CDK inhibitors are best suited to all cells, synchronizing at a specific cell cycle phase. At present, CDK inhibitors are best suited in anti-cancer drug discovery laboratories.***

6.3.4 Synchronization of Cells in the G2 Phase of Cell Cycle

Inhibition of Microtubule Formation and G2 Arrest

Microtubules (made up of **tubulin proteins**) are one of the most important components of the **cytoskeleton** involved in cell division. They are necessary for the segregation or separation and distribution of the chromatids/chromosomes across the two ends of a dividing mother cell. Eventually, this mother cell will be divided into two daughter cells. Therefore, microtubules are very important drug targets to control the cell cycle. At present, several microtubule inhibitors are used that synchronize the cells at the G2-M transition. Experimentally, the most widely used microtubule inhibitors are **colcemid, nocodazole, paclitaxel, vincristine, and vinblastine**.

Inhibition of Cdk1 and G2 Arrest

The G2 phase of the cell cycle uses different cyclins (e.g., cyclin B) and CDKs (e.g., CDK 1). In a true sense, these cyclins and CDKs work along the G2-M phase boundary, leading to the transition of cells from the G2 to M phase of the cell cycle. The scientific community across the world has developed several CDK1 inhibitors. For example, a small molecule inhibitor, **RO-3306** (Roche), can arrest cells in the G2 phase. As indicated by the developers, the success of CDK inhibitors depends on several factors, including the cell line or cell type used, the right concentration, and optimum incubation time.

NB: A comparative scientific preference of microtubule inhibitors versus CDK 1 inhibitors claimed CDK1 inhibitors as the preferred experimental materials of interest. Most probably, this is because CDK inhibitors are generally less toxic than microtubule inhibitors, which allows the scientists to further use the cells as per the experimental requirement(s).

S Phase Synchronization

During the S phase, the replication of DNA takes place, and therefore the number of chromosomes is doubled, a prerequisite for mitosis. One of the most well-studied S phase regulators is **2[[3-(2,3-dichloro phenoxy) propyl] amino]ethanol (2,3-DCPE)**. This molecule works by activating certain **caspases** ***(a family of cysteine proteases involved in apoptosis)*** and **reducing the BCL-XL** ***(a BCL member family protein involved in antiapoptotic activities)*** **protein formation**.

6.3.5 Confirmation of Cell Synchronization

Experimental conformation mandates documentation and confirmation of cell synchronization at a specific cell cycle phase. Either **microscopy** or **flow cytometry** is used to ascertain cell synchronization at a particular cell cycle phase. Best utilization would be via phase-specific fluorescent-labeled antibodies. The protocols for microscopy or flow cytometry are discussed in their further, specialized chapters.

7 Concept of Mammalian Cell and Tissue Culture

- **Mammalian cell culture** is defined as the process by which cells are isolated from a mammalian body (specific organs or tissues) and are grown in vitro under controlled laboratory conditions of maintained **pH, temperature, humidity, O_2 tension, and readymade nutrient supply** (through cell culture medium).
- **Tissue culture** is a collective process by which whole tissues are isolated from a mammalian organ and are subsequently grown in vitro in controlled environmental conditions, either on semi-solid, solid (**aga**r), or liquid (**nutrient broth**), supplemented with nutrients and growth factors, similar to the cell culture medium.
- The term tissue culture refers to the culturing of tissue pieces or parts of an organ isolated from any plant or animal cell or organ, i.e., **explant culture**.

- The controlled laboratory conditions like pH, temperature, and humidity of the growth medium are optimized using **a CO_2 incubator**.
- **Nowadays, the term tissue culture is often used interchangeably with cell culture.**
- Different cells such as **endothelial cells, fibroblasts, smooth muscle cells, cardiac muscles, epithelial cells**, etc. present in various mammalian organs such as the **liver, breast, prostate, ovary, skin, kidney**, etc. are grown in cell culture. The specific requirement of culture medium for these cells is attained through the requisite inclusion of hormones, growth factors, specific ECM proteins, neurotransmitters, etc.
- Besides the above-mentioned, **normal mortal cells** that originated from various organs, cells from many **tumors or cancers** can also be grown in cell/tissue culture.
- Cells are taken from normal or tumor/cancer-affected tissues/organs and are generally grown as adherent cultures.
- Additionally, cells (particularly leukocytes) present in the normal or cancer-affected blood cells can also grow in **suspension culture** (Phelan 1996; Acheson 1993; Rodríguez-Hernández et al. 2014).

Here is a summary of major discoveries in the field of cell/tissue/organ culture:

8 Historical Events in the Development of Mammalian Cell Culture Technology

- Over the last 200 years, a large number of scientists have worked very hard to develop cell, tissue, and organ culture protocols.
- In early **1878**, physiologist **Claude Bernard** was the first to propose in vitro maintenance of live cells or tissues via intact physiological functions, even after an animal's death.
- In **1907**, the term "Cell Culture" was first successfully postulated by **Ross Harrison, presently remembered as the "Father of Cell Culture."**
- Mammalian cell culture became more popular in the nineteenth century after the discovery of a **chemically defined cell culture medium** by **Lewis and Lewis** for the supply of specific nutrients facilitating culturing of a particular cell type.
- At the same time, (**~1928**) invention of the **antibiotic Penicillin** by Nobel laureate **Alexander Flemming** enabled the arresting of pathogenic microbe growth (especially bacteria) in cell culture.
- **The discovery of antibiotic penicillin from *Penicillium notatum,* yeast was one of the major achievements of the contamination-free mammalian cell culture.**
- In the **mid-1900s,** with the breakthrough discovery of proteolytic enzyme **trypsin**, both cell and tissue culture emerged as essential **techniques** in modern biology laboratories. Cells that are attached to the culture containers **(adherent cells**) could now be easily detached from the surface of culture flasks for

harvesting using proteolytic enzymes such as trypsin. These harvested cells are subsequently transferred to a new culture flask with a fresh medium for **further propagation**. This technique is commonly called a **subculture** of cells.

- In terms of **two-dimensional (2D)** cell culture containers, the major discoveries were by **Julius Richard Petri (the discoverer of the Petri dish)** and **Carrel and Baker** (the discoverers of the **T flask)**.
- Other major discoveries in the mammalian cell culture were the mammalian cell culture in **three dimensions (3D),** the discovery of various instruments *(*e.g., **Bioreactors**) for the large-scale culture of mammalian cells to enable the production of various molecules such as recombinant proteins, vaccines against viruses, enzymes, etc. on an industrial scale. The basic mammalian cell culture techniques now began to be used for **organ, organotypic and histotypic cultures.**
- Besides various **human cells (originated either from healthy or diseased organs such as tumors and cancers)**, both **embryonic and adult mouse cells** (being an inexpensive and reproducible source of biological experiments) could be used for mammalian cell culture regularly. Other mammalian cells that originated from **rats, monkeys, or guinea pigs** also emerged as a provision for large-scale cell culturing (Dumont et al. 2016; Jedrzejczak-Silick 2017).
- ***Here is a chronological list of events as the major discoveries in the field of cell/tissue/organ culture:***
- **1595**: ***Zacharias Janssen*** *and his father* ***Hans*** is credited with the invention of the first compound microscope.
- **1655**: ***Robert Hooke*** described "**cells**" in **cork**.
- **1674–1676**: ***Anton Van Leeuwenhoek*** discovered protozoa. He observed bacteria some 9 years later.
- **1833**: ***Robert Brown*** described the cell **nucleus** in orchid cells.
- **1838**: ***Schleiden*** *and* ***Schwann*** proposed **cell theory**.
- **1840**: ***Albrecht von Roelliker*** realized that sperm and egg are nothing but cells.
- **1856**: ***Pringsheim N***. observed how a sperm cell penetrated an egg.
- **1855–1858**: ***Rudolf Virchow*** (physician, pathologist, and anthropologist) expounds his famous conclusion: *Omniscellulaecellula*, that is **cells develop only from pre-existing cells** [cells come from pre-existing cells].
- **1857**: ***Albert von Kolliker*** described **mitochondria for the first time (*Carl Benda*, in 1898, used the term mitochondria).**
- **1877: *Julius Richard Petri*** discovered Petri Dish.
- **1878: *Claude Bernard*** proposed that the physiological systems of an organism can be maintained in a living state even after the organism's death.
- **1880: *Amoled*** showed that leukocytes can divide outside the body.
- **1882: *Walter Flemming*** discovered the process of cell division.
- **1885: *Wilhelm Roux*** illustrated the maintenance of embryonic chick cells in a saline culture.
- **1897: *Loeb*** demonstrated the survival of cells isolated from blood and connective tissue in serum and plasma.
- **1898: *Camillo Golgi*** described the Golgi apparatus.

- **1898:** ***Liunggren*** for the first time maintained human tissue (skin) in vitro in the ascetic fluid.
- **Nineteenth century:** ***Sydney Ringer*** developed salt solutions containing the chlorides of sodium, potassium, calcium, and magnesium, suitable for maintaining the functioning of an isolated animal heart outside the body.
- **1903:** ***Jolly*** studied the behavior of animal cells immersed in serum lymph (**Also observed cell division of salamander leukocyte)**.
- **1907:** ***Ross Granville Harisson*** cultivated frog nerve cells in lymph clots and observed the growth of nerve fiber in vitro. He is considered then **"Father of cell culture."**
- **1910:** ***Burrows*** succeeded in the long-term cultivation of chicken embryo cells using the **plasma clot method**. He made detailed observations of mitosis.
- **1911*: Lewis and Lewis*** made the **first liquid cell culture medium** consisting of sea water, serum, embryo extract, salts, and peptones. They observed limited monolayer growth.
- **1912–1913:** ***Carrel*** introduced strict aseptic techniques so that cells could be cultured for long periods.
- **1916:** ***Rous and Jones*** introduced proteolytic enzyme **trypsin** for the subculture of adherent cells.
- **1920:** The ***ECACC*** is established for cell culture preservation.
- **1922:** ***Albert Ebeling*** cultured epithelial cells for the first time.
- **1923:** ***Carrel and Baker*** developed "Carrel" or **T-flask** as the first specifically designed cell culture vessel. They studied microscopic observations of cells in culture.
- **1923:** ***Carrel and Ebeling*** performed a subculture of the fibroblastic cell line.
- **1924:** ***Levitsky for the first time,*** used the term **karyotyping**.
- **1925:** The ***ATCC*** is established for cell culture technique evaluation.
- **1925–1926**: ***Strangeweys and Fell*** discovered cell differentiation through in vitro organ culture.
- **1926:** ***Svedberg*** developed the first analytical ultracentrifuge.
- **1920–1930:** ***Carrel and Ebeling*** **subcultured the fibroblast cell line for the first time**.
- **1927:** ***Carrel and Rivera*** produced the first viral vaccine: Vaccinia.
- **1928:** ***Alexander Flemming*** discovered penicillin from *Penicillium notatum.*
- **1930:** ***Carrel and Linberg's*** new cell culture devices discovery.
- **1933:** ***George Gey*** developed the **roller tube technique** for cell culture.
- **1938:** ***Behrens*** used differential centrifugation to separate nuclei from the cytoplasm.
- **1943:** ***Earle*** *and coworkers* established the L-cell mouse fibroblast cell line, the first-ever continuous cell line.
- **1948:** ***Keilova, Cruikshank, and Lowbury*** introduced **antibiotics** in cell culture.
- **1948:** ***Sanford*** derived clone 929 from L cell line.
- **1948:** ***Fischer*** developed a chemically defined medium, **CMRL 1066.**
- **1949:** ***Enders*** established the growth of a virus in cell culture.

- **1951–1952:** ***George Gey*** cultured continuous human cancer cell line (**HeLa**) from human cervical carcinoma for the first time.
- **1952:** ***Kew*** *and coworkers* reported ***polio virus*** culturing for the first time in monkey kidney cells.
- **1952:** ***Dulbecco*** developed a plaque assay for animal viruses using cultured cell confluent monolayers.
- **1953:** ***Crick, Wilkins, and Watson*** proposed a **double-helical DNA structure**.
- **1954:** ***Abercrombie*** observed contact inhibition through ceased motility of diploid cells in monolayer culture upon establishing contact with adjacent cells.
- **1955:** ***Eagle*** studied the nutrient requirements of selected culture cells and established the first widely used **chemically defined medium**.
- **1856–1857:** ***Louis Pasteur*** **for the first time showed that lactic acid fermentation is caused by a living organism**.
- **1957:** ***Meselson, Stahl,*** *and* ***Vinograd*** developed density gradient centrifugation in cesium chloride solutions for separating the nucleic acids.
- **1958:** ***Coriell*** recognized the role of mycoplasma in cell culture contamination.
- **1959:** ***Puck Marcus*** conducted the cloning of HeLa cells on a homologous feeder layer.
- **1961:** ***Sorieul*** *and* ***Ephrussi*** discovered cell fusion and s**omatic cell hybridization**.
- **1961:** ***Hayflick*** *and* ***Moorhead*** isolated human fibroblasts (WI-38), demonstrating their finite lifespan in culture, famously known as the **Hayflick** effect.
- **1962:** ***Macpherson*** *and* ***Stoker***'s establishment and transformation of BHK21 cells.
- **1964:** ***Klein Smith*** *and* ***Pierce*** discovered the pluripotency of embryonic stem cells.
- **1964:** ***Littlefield*** introduced the HAT medium for cell selection.
- **1965:** ***Ham*** introduced the **serum-free medium**, which was able to support the growth of some cells. Discovered serum-free cloning of **Chinese Hamster Ovary** (**CHO**) cells.
- **1965:** ***Harris*** *and* ***Watkins*** were able to fuse human and mouse cells using a virus.
- **1967:** ***Hoover*** *and* ***Cohen*** discovered epidermal growth factor (**EGF**).
- **1968:** ***Stoker*** *and coworkers* established anchorage-independent **microbial growth**.
- **1969:** ***Metcalf*** discovered colony formation in hematopoietic cells.
- **1970:** ***Kruse*** *and coworkers* developed the first-ever **laminar flow cabinets for cell culture**.
- **1975:** ***Kohler*** *and* ***Milstein*** produced the first **hybridoma cells** capable of secreting a **monoclonal antibody.**
- **1976:** ***llmensee*** *and* ***Mintz*** established the **totipotency** (the capacity to proliferate and differentiate into a complete body) of embryonic stem cells.
- **1976:** ***Hayashi*** *and* ***Sato*** prepared growth factor-supplemented **serum-free medium** for the first time.

- **1976:** ***Sato*** *and colleagues* published papers showing that different cell lines require different proportions of hormones and growth factors in serum-free media.
- **1977:** ***Nelson-Rees*** *and* ***Flandermeyer*** confirmed cross-contamination of many cells through HeLa cells.
- **1978:** ***Ham*** *and* ***McKeehan*** prepared MCDB selective serum-free medium.
- **1978: Sato** established the basis for the development of serum-free media from cocktails of hormones and growth factors.
- **1978:** ***Prindull G.*** *and coworkers* (***N. Van Der Meulen***) discovered stem cells in human cord blood.
- **1980–1987:** ***Peehl*** *and* ***Ham, Hammond,*** *and* ***Kendler*** developed many specialized cell lines.
- **1981:** ***Martin*** *and coworkers* developed in vitro stem cell lines from mice for the first time.
- **1982:** ***Genentech*** licensed human insulin as a recombinant protein for therapeutic purposes for the first time.
- **1982: Kleinman H.K**. *and others* first **described the use of matrigel and other materials for 3D culture.**
- **1983:** ***Bell*** *and others* developed reconstituted cell cultures.
- **1984:** ***Collen*** illustrated the development of recombinant tissue-type plasminogen activator in mammalian cells.
- **1983–1985:** ***Genentech*** produced human growth hormone from recombinant bacteria, subsequently accepted for therapeutic use.
- **1986:** Licensing of Lymphoblastoid γ-IFN (gamma interferon).
- **1987:** Doctors ***Mario R. Capecchi, Martin J. Evans, and Oliver Smithies*** **created the first-ever knockout mouse.**
- **1987:** Commercial availability of tissue-type plasminogen activator (tPA) from recombinant animal cells.
- **1989:** ***Weinberg*** explained **oncogenes, malignancy, and transformation.**
- **1989:** ***Kleinman KS*** and coworkers used recombinant erythropoietin protein on clinical patients (a clinical trial) for the first time.
- **1990:** Recombinant products in a clinical trial (HBsAG, factor VIII, HIVgp120, CD4, GM-CSF, EGF, mAbs, IL-2), were postulated.
- **1992:** ***SkinEthics*** produced human tissue and neural stem cell cultures in vitro.
- **1995:** ***Thomsom JA*** *and coworkers* developed an embryonic stem cell line from a primate for the first time.
- **1996:** ***Willmut*** *and coworkers* created the first **transgenic sheep, Dolly,** through the nuclear transfer technique (announced in 1997).
- **1997:** PPL Therapeutics produced a transgenic **lamb, Polly.**
- **1998:** ***Teruhiko Wakayama*** and coworkers cloned mice from somatic cells.
- **1998:** ***Aigner*** produced cartilage using tissue-engineered cell culture.
- **1998:** ***Thomson and Gearhart*** isolated and cultured the human embryonic stem cells.
- **1999**: ***Hamilton*** *and* ***Baulcombe*** discovered **si-RNA** as part of post-transcriptional gene silencing (PTGS) in plants.

- **2000:** ***Dennis*** *and coworkers* formulated a **human genome project** along with genomics, proteomics, genetic deficiency, and expression errors.
- **2000:** ***Keril*** established a robust cell-based system for genetic and functional analysis of HCV replication in culture.
- **2000:** ***Kwon*** established the rapid cell sheet detachment from **polyisopropyl acrylamide-grafted porous cell culture membranes**.
- **2000:** ***Benjamin*** described the derivation of **ES cells** from human blastocysts for somatic differentiation in vitro.
- **2001:** ***Edna*** *and others* described the 3D matrix composition and function of adhesion, derived from tissue or cell culture.
- **2001:** ***Nicole*** *and others* showed enhanced hepatitis C virus RNA replication by cell-culture adaptive mutations.
- **2001:** ***Chu*** *and* ***Robinson*** established industrial choices of protein production by large-scale **cell culture** using standard **stirred tank reactors**.
- **2001:** ***Potter*** *and* ***DeMarse*** developed a new method for cell culturing that maintains their health and sterility for many months.
- **2001:** ***Keefer*** *and others* **cloned goats** by nuclear transfer of **adult somatic cells**.
- **2002:** ***Brinster*** developed the testis cell transplantation method, providing a powerful approach to studying the biology of the male germline stem cells and their specific environment via stem cell niche.
- **2002:** Human Genome Society of France claimed the production of a **cloned human baby**, named Eve (Clonaid).
- **2002:** ***Shao*** *and others* described the SILAC method for stable isotope labeling of amino acids in cell culture for in vitro incorporation of specific amino acids into all mammalian proteins.
- **2002:** ***Atala and Lonza*** exploit tissue engineering.
- **2003:** ***Park*** *and* ***Shuler*** integrated cell culture with **micro-fabrication technology**.
- **2004:** ***Cowan*** and colleagues showed that the adipose tissue-derived adult stromal cells healed critical-sized mouse calvarial defects and skeletal defects without genetic manipulation or the addition of exogenous growth factors.
- **2004:** ***Kunz Schughart*** *and colleagues* discovered the use of **3D cell culture** for high throughput screening: **the multicellular** spheroid model detects specific cellular effects reflecting actions on various targets.
- **2004:** ***Sin*** *and coworkers* developed the three chambers of **micro-scale cell culture analog device** with integrated dissolved oxygen sensors. The method provides an in vitro supplement to animal studies with a possibility of a human surrogate for predicting human response in clinical trials.
- **2005:** ***Fulcher*** *and coworkers* described for the first time the culture of well-differentiated human airway epithelial cells.
- **2005:** ***Jonathan*** *and others* described the computer-controlled **microcirculatory support system** for endothelial cell culture.
- **2005:** ***Wang*** *and colleagues* postulated the **application of bioreactors as** powerful tools for large-scale mammalian cell culture.
- **2005:** ***Xu*** *and others* discovered **inkjet printing** of viable mammalian cells.

- **2005:** ***Yaakov*** *and others* discovered laser-guided **direct writing for 3D tissue engineering**, with underlying processes such as liver and pancreas morphogenesis, differentiation, and angiogenesis.
- **2006:** ***Shah*** *and colleagues* discovered **nucleo-counter**, an efficient technique for determination of the cell number and viability in the animal cell culture process.
- **2006:** ***Yamanaka*** obtains induced pluripotent stem cells.
- **2007:** ***Pampaloni*** *and others* described that 3D culturing bridges the gap between cell culture and live tissues. They illustrated a strong impact of 3D culture on drug screening and the use of laboratory animals for experimental purposes.
- **2007:** ***Yu*** *and coworkers* used viral vectors to reprogram adult cells to an embryonic state (**induced pluripotent stem cells**).
- **2007:** ***Parker*** *and* ***Townley*** described the biomimetic properties of **photonic nanostructures** in cell culture.
- **2008***:* ***Smalley*** *and colleagues* described a 3D cell culture model for the first time.
- **2008** ***and beyond***- Era of induced pluripotent stem cells in therapy-ongoing promises and challenges.
- **2009:** ***Fernandes*** *and others* described the scale-up of human embryonic stem cell culture in a stirred **micro-carrier system**.
- **2010:** ***Barbulovic-Nad*** *and colleagues* described a **microfluidic platform** for complete mammalian cell culture – the automated cell micro-culture technique.
- **2010+** ***Atala demonstrates*** 3D bioprinting techniques for tissue and organs.
- **2013:** ***Castro*** *and others* illustrated the high-throughput single nucleotide polymorphism (**SNP)-based authentication of human cell lines** (a solution for misidentification of cell lines).
- **2013:** ***Dayeh*** *and others* proposed the use of fish-derived cell lines for the determination of environmental contaminants.
- **2013:** ***Kalos*** *and* ***June*** **proposed the adoptive T cell transfer for cancer immunotherapy** in the synthetic biology era.
- **2013:** ***Kanatsu Shinohara*** *and* ***Takash*** discovered spermatogonial stem cell renewal and development, enabling the use of these cells for application in animal transgenesis and medicine.
- **2013:** ***Lee*** *and colleagues* described the photostable fluorescent organic dot with aggregation-induced emission for noninvasive long-term tracing.
- **2014:** ***Landuer*** designed the medium for industrial-scale CHO cell culture.
- **2015:** ***Takahashi*** described the gene transfer into cultured mammalian embryos through electroporation.

9 Selection and Validation of Appropriate Mammalian Cells for Culture and Experiments

- As discussed in the earlier paragraphs, the mammalian body is made up of two types of cells, i.e., somatic cells and germ or reproductive cells. In a mammalian body, all the somatic cells are genetically the same.

- It was also discussed previously that within a cell at a particular time, while certain genes are **transcriptionally switched on**, others may be **transcriptionally switched off**. This switching on or switching off of genes (**transcriptional regulation**) in somatic cells differs from one cell type to another.
- Thus, **due to differential expression** patterns, **mRNAs** and **proteins** expressed by one type of somatic cell may vary in another type of somatic cell. Changes in the protein expression patterns lead to **structural (phenotypic)** and **functional** variations from one another type of somatic cells.
- So, every somatic cell has its own structure and functions. For example, neuronal cells look like a tree and are involved in nerve conduction, and smooth muscle cells look like a spindle and are involved in involuntary muscle contraction.
- All cell lines, whether normal (having a definite life span) or immortal/transformed (growing continuously), originate from a normal mammalian cell.
- All the cell lines, whether normal/mortal or immortal (tumor/cancer) can be characterized based on their origin, except **anaplastic cells**, which change vibrantly (a high level of mutation occurs in these cells), it is difficult to identify their originating cells.

Thus, it validates and necessitates selecting or choosing a particular type of somatic cell or cell line for the very purpose of culturing and examining a particular structure/function related to this particular cell line.

- A large number of databases are nowadays available to wisely select the particular cell line for culture and research (Amirkia and Qiubao 2012).

The selection and validation of an appropriate mammalian cell line can be explained by discussing the following points:

1. Process of Selection of Appropriate Cells for Culture
2. Validity and Authenticity of the Chosen Cells
3. Importance of Working with Low Passage Number Cells
4. Examine Whether the Chosen Cells Are Contamination Free
5. Examine Whether the Chosen Cells Exhibit the Right Biology
6. Examine Whether the Chosen Cells Are Suitable for the Desired Experiments

Here is a brief explanation of the above points:

9.1 Process of Selection of Appropriate Cells for Culture

- At the outset, we need to understand why we are culturing mammalian cells. For example, if we are examining the expression and function of a general protein (such as glyceraldehyde-3-phosphate dehydrogenase, or GAPDH, a protein involved in glycolysis) for basic research, we can choose any mammalian cell

for culture. This is because GAPDH is a glycolytic enzyme and is expressed by all metabolic cells of a mammalian body.

- If our purpose is to produce a large amount of a particular protein for industrial purposes, we need to check what kind of cell may be suitable for our culture. For example, **CHO or HEK-293 cells** are extensively used in the **bioprocessing industry** for the production of various proteins or enzymes because of their specific capacity for mammalian-specific post-translational modifications, particularly glycosylation of proteins.
- If our purpose is to monitor the responsive behavior of cancer cells under some specific biochemical conditions, we urgently require the selection of a cancer cell in a culture medium that may be best fitted to our interest.
- If our purpose is more specific, for example, to check the role of estrogen on breast cancer complications, we must select a breast cancer cell line (e.g., **MCF-7 cell line**) that expresses estrogen receptors **(ERs**) and then the culture or treat them with various estrogens.

To confirm whether the cell line we have chosen is suitable for our experiment or not, we need to compare the biomarker expression and its specific expression extent through the literature survey.

9.2 Validity and Authenticity of the Chosen Cells

- Working with more than one cell line simultaneously may accidentally **cross-contaminate** one cell with the other. For example, several reports suggest that **HeLa cells** can easily contaminate other cells if cultured at the same time. Isolated from aggressive cervical adenocarcinoma, these cells have emerged as one of the most probable contaminants for more than the last 50 to 70 years. Studies voiced such contaminations through DNA **fingerprinting of short tandem repeats (STRs)** at selected locations, wherein one investigation noticed an indistinguishable fingerprint of the human endometrial cell line, HES, and the HeLa cells. Reports from international cell banks reveal that cell line improper identification was 16% in 1977 and increased to 18% in 1988, suggesting the persistence of this problem over a decade. Although HeLa cells remain major contamination suspects, the menace of contamination is not limited to them, if the observations from ATCC are to be believed. The data bank claims cross-contamination of continuous cell lines to be a pervasive issue in biomedical research, wherein one instance found that a human epithelial cell line immortalized by human telomerase reverse transcriptase overexpression is nothing but the human breast cancer cell line, MCF-7. The importance of this cross-contamination has been a menace, and it was only this troubling bottleneck that laid the basis for cell line authentication through the introduction of **genetic markers by *Gartler* in 1967** (Gartler 1967).
- Henceforth, many variations include inspection of banded marker chromosomes and visualization of chromosomal pattern and architecture, Human Leucocyte

Antigen (**HLA) typing**, enzyme polymorphisms, DNA polymorphisms, and relatively recent, DNA fingerprinting and use of locus-specific probes (Lee 1991).

- Another problem of working with more than one cell line is **labeling mistakes** during cell culture and experiments.
- Further, during storing of cells in an ultra-cold (liquid nitrogen) environment, **labeling may be removed** as there are significant chances of misidentification.
- The database is now available for **"Cross-contaminated or Misidentified Cell Lines."** Here is one of the important web pages:
  http://standards.atcc.org/kwspub/home/the_international_cell_line_authentication_committee-iclac_/Database_of_Cross_Contaminated_or_Misidentified_Cell_Lines.pdf

So, authentication of the mammalian cell line is indeed a crucial step in mammalian cell culture.

- It is claimed that cell banks like the American Type Culture Collection (**ATCC**), the German Collection of Microorganisms and Cell Cultures/**DSMZ**, the European Collection of Cell Cultures (**ECACC**), the Japanese Collection of Research Bio-resources (**JCRB**), the RIKEN Bio-resource Center Cell Bank/ **RIKEN** properly **authenticate** and **quality control** the cell line(s) before sending it/them to the probable buyer (Hay 1992).
- Many methods, such as **isoenzyme analysis, karyotyping, human lymphocyte antigen (HLA) typing,** and characterization of **amplified fragment length polymorphisms (AFLP),** have been used to identify cross-contamination in cell culture.
- However, a superior method is **Short Tandem Repeat (STR)** profiling, which is well-established for identification and confirmation of a mammalian cell line and DNA-based forensic identification. STR loci consist of short, repetitive sequence elements (3–7) base pairs in length. STR may have the following repetitive sequences: **Pentanucleotide:** AAAAGA AAAGAAAAGAAAAGA; **Tetranucleotide:** AAAG AAAGAAAGAAAG; **Trinucleotide:** CTT CTTCT TCTTCTT; **Dinucleotide:** AG AGAGAGAGAG (Master et al. 2001).
- The number of repeats in STR loci can be highly variable among individuals, which makes these genetic markers effective for **human identification** or **cell line authentication purposes**. STRs have become **popular DNA repeat markers** because they are easily amplified by the polymerase chain reaction **(PCR)** using PCR primers that bind to the flanking regions surrounding the STR repeat. Multiple STR loci can be examined simultaneously to create a DNA profile. Generally, **Multiplex Real-time PCR is the best method to confirm STR** (Cabrera et al. 2006; Liscovitch and Ravid 2006; Cooper et al. 2007).

9.3 Importance of Working in Low Passage Number Cells

- When a normal (mortal) cell is cultured again and again through several passaging, it will start losing many of its characteristics due to **aging/senescence**.

Finally, every mortal cell must lose the capacity of the division after a certain number of divisions and ultimately perishes ***(Heyflick effect)***.

- For example, after 4 to 5 passages, primary cultured endothelial cells start losing estrogen receptors, and after around 15 passages, these cells lose the majority of their estrogen receptors.
- Although, in general, endothelial cells have the capacity of around **50 generations**.
- Another important problem in the continuous passaging of mammalian cells is the genetic drift leading to the alteration of several changes in the growing cell line.
- Not only normal cells but even **some cancer cells** exhibit genetic drifting. Before culturing, one must check instructions from the supplier (e.g., **ATCC**), such as how many continuous passages of a cell line are possible without any genetic drifting.
- Therefore, if somebody's purpose is to check the effect of estrogens on primary cultured endothelial cells, we must choose early passaged primary cultured endothelial cells. If our purpose is to ascertain the cell proliferation and cell cycle, the cancer cell may be chosen because most cancer **cells have** no **or short G_o phase that leads to continuous division and accumulation** of a large **number of cells in a relatively short duration.**

9.4 Examine Whether the Chosen Cells Are Contamination-Free

- The serum is one of the growth-promoting agents used in mammalian cell culture. For mammalian cell culture (depending upon the type of cells), 5–20% of fetal calf serum (**FCS**) or fetal bovine serum (**FBS**) is generally used. **The serum is the most prominent source of contamination with mycoplasma, which cannot be removed by filtration sterilization because of its small size (mycoplasma is claimed to be the smallest living creature on this planet earth)**. **Trusted cell sources will supply serum/cell stocks as verified mycoplasma-free** (McGarrity et al. 1984; Lincoln and Gabridge 1998).
- Additionally, during routine culture or subculture, cells may get contaminated with **bacteria, fungus,** and other microorganisms that are present in the environment (**air/water/floors of the laboratory/on the instruments**, **virtually everywhere**). The contaminated microorganism can spoil the cell culture because of its relatively **short doubling time (e.g., *E coli* may have a doubling time of just 20 min)**. Various pathogenic viruses are other sources of contamination, particularly cell biologists should be aware of the genetically transmitted viruses (Weiss 1978).
- If the contamination status of a cell stock is not known, it is good practice to begin culturing in a **quarantine environment** and establish a **"clean" status** before moving into general circulation. Therefore, every effort should be made to get a contamination-free culture. Chapter of this book entitled, ► "Microbial Contamination of Mammalian Cell Culture" separately discusses the contamination-associated problems in mammalian cell culture.

9.5 Examine Whether the Chosen Cells Exhibit the Right Biology

- **The Cancer Line Encyclopedia:** A chosen cell line must fulfill its biological functions as per the knowledge acquired from the literature. For example, the **cancer line encyclopedia (CCLE**) represents around 1000 cancer cell lines. This representation includes the basic knowledge about various established cancer cell lines, their origin, specific genetic aberrations (mutations) related to various cell lines, markers to identify a specific cell line, and so on. These details help the researchers to select specific cell lines of interest.
- **The Catalogue of Somatic Mutations in Cancer: The catalogue of Somatic Mutations in Cancer (COSMIC)** is an excellent resource for somatic mutations of several cancer cell lines.
- Besides applied biology such as cancer, one may choose to work on basic cell biology and physiology. So, based on the specific need, cell lines must be selected. For example, endothelial cell lines, as well as primary cultured endothelial cells would be the specific choice of interest if somebody intends to work on both, the physiology of vasomotion as well as the pathophysiology of atherosclerosis.

9.6 Examine Whether the Chosen Cells Are Suitable for Desired Experiments

- Every cell line has its morphology (**phenotype**) and a few characteristic biological activities. Morphology and biological functions depend on the cell culture medium composition and culture conditions, which again influence the growth rate, transfection ability (e.g., strictly adherent cells such as endothelial cells exhibit a low transfection rate), and other cellular characteristics. Thus, several factors influence the selection of cell lines for specific experimental purposes.
- Cost-effectiveness (for handling, maintenance, and experimental use) is one of the most important factors that may influence the selection of a particular cell line.
- For example, while induced pluripotent stem cells (**iPSCs**) may represent a more "normal" model than a cancer cell line, the culture and maintenance costs of iPSCs prevent their use in some laboratories.

NB: American Type Culture Collection (ATCC) has the world's largest and most extensive database of various cell lines, including more than 4000 continuous cell lines. The database includes species, the tissue/organ-specific origin of the cell lines, and specific signaling pathways involved in both physiology and pathophysiology.

10 Conclusions

This introductory first chapter describes the process of formation of the mammalian body through **cells**, **tissues, organs, and organ systems**. This chapter primarily focuses on **cell theory,** besides the identification and characterization of cells via karyotyping and cell morphology. As the chapter goes forward, the process of mammalian **cell division,** i.e., mitosis and meiosis are discussed very briefly. The topic of cell division is addressed, as cell division is the fundamental key process for increasing the number of cells or cell proliferation. Finally, the main topic of this introductory chapter, i.e., the basic concept of mammalian cell culture, has been discussed. Once again, **mammalian cell culture** is defined as the process by which cells are isolated from mammalian tissues or organs and are grown in vitro in readymade synthetic nutrients under controlled laboratory conditions of maintained pH, temperature, and humidity in either a CO_2 incubator or in an industrial-scale Bioreactor. Today, **the terms cell culture and tissue culture are often used interchangeably**. To understand the various aspects of cellular physiology, particularly the effects of various drugs/toxins, etc. on the cultured cells, **synchronization** of different cultured cells is highly essential. Synchronization is a particular state in which all the cells remain simultaneously in the desired phase (**G1-S-G2-M**) of the **cell cycle.** The most desired phase to stop the cell cycle is the G1 phase since the G1-S transition is responsible for effectively initiating the cell cycle. The easiest way to stop the cells at the G1 phase of the cell cycle is **serum starvation**. As an introductory chapter, age-old discoveries of various technological aspects of mammalian cell culture by different scientists are mentioned carefully. The chapter finally addresses the process of validation and authentication of chosen mammalian cells. This section of the chapter mainly addresses six major points, i.e., (1) Process of selecting appropriate cells for the initiation of culture; (2) Validity and authenticity of the chosen cells; (3) Importance of working with the cells having a low passage number; (4) Examine and monitor any microbial or chemical contamination in the chosen cells; (5) Confirm whether the chosen cells exhibit the right morphology and physiology; (6) Determine whether the chosen cells are suitable for desired experiments**.** The chapter ends with the identification and validation of the chosen cells before commencing the cell culture.

11 Cross-References

- ▶ Bioimaging: Usefulness in Modern Day Research
- ▶ Biosensors' Utility in Mammalian Cell Culturing
- ▶ Common Reagents and Medium for Mammalian Cell Culture
- ▶ Culture of Continuous Cell Lines
- ▶ Culture of Neuron and Glia Cells
- ▶ Emerging Drug Delivery Potential of Gold and Silver Nanoparticles to Lung and Breast Cancers
- ▶ Establishment of a Cell Culture Laboratory

- Experimental Mammalian Cell Culture-Based Assays
- Isolation and Purification of Various Mammalian Cells: Single Cell Isolation
- Isolation and Primary Culture of Various Mammalian Cells
- Large-Scale Culture of Mammalian Cells for Various Industrial Purposes
- Mammalian Cell Culture in Three Dimensions: Basic Guidelines
- Mammalian Cell Culture Laboratory: Equipment and Other Materials
- Mammalian Cell Culture Types and Guidelines of Their Maintenance
- Mammalian Cells: Reliability as Model System in the Ecotoxicological Evaluation of Environmental Stressors
- Mammalian Cells, Tissues and Organ Culture: Applications
- Microbial Contamination of Mammalian Cell Culture
- Nanomaterials: Compatibility Towards Biological Interactions
- Organ, Histotypic and Organotypic Culture, and Tissue Engineering
- Primary Culture of Immunological Cells
- Stem Cell Culture and Its Applications
- Troubleshooting of Mammalian Cell Culture

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