



# **Lectures of Ultrastructure of Cell**

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**By**

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## **Cell and Tissue Culture**

The pioneers in this technique, who removed cells and tissue, kept them viable, and were able to make them multiply and grow, had a revolutionary impact on science. Their work gave birth to the new discipline of cell biology, which has advanced almost explosively during the past half century. Cell biology advances have gone hand in hand with the ever-increasing ability to isolate, maintain, and examine increasing numbers of cell types.

In the organism (in vivo), cells are bathed in fluid derived from blood plasma and containing many different molecules required for survival and growth, while the term of cell culture refers to the removal of cells from an organism and their subsequent growth in a favorable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation. Cell culture allows the direct observation of cellular behavior under a microscope and many experiments technically impossible to perform in the intact animal can be accomplished in vitro.

### **Culture Conditions**

Culture conditions vary widely for each cell type, but the artificial environment in which the cells are cultured invariably consists of a suitable vessel containing a substrate or medium that supplies the essential nutrients (complex solutions of known composition: salts, amino acids, carbohydrates, vitamins, minerals), specific growth factors, and regulates the physicochemical environment (pH, osmotic pressure, temperature, O<sub>2</sub>, CO<sub>2</sub>).

Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as senescence; these cell lines are known as finite. However, some cell lines become immortal through a process called transformation, which can occur spontaneously (or some related to oncogenes) or can be chemically or virally induced. When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line.

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Some cells can be maintained in vitro for long periods because they become immortalized and constitute a permanent cell line. Improvements in culture technology and use of specific growth factors now allow most cell types to be maintained in vitro.

**Procedure for production of a cell line**

1. A piece of tissue is removed from an organism.
2. Adhesion between cells is broken by enzymes like trypsin or collagenase.
3. Cells are transferred to a plastic dish or bottle which contains cultured medium.
4. Cells are incubated at 37°C in 5-10% CO<sub>2</sub> and 90-95% O<sub>2</sub>.
5. Cells grow, divide and cover the surface of the container; this culture is referred to as primary cell culture; all cells will stop dividing due to contact inhibition.
6. Cells are transferred to a fresh medium and will again start growing. This type of repetitive culturing of the cells is limited, why? Because the growth of animal cells ceases after about 50 divisions, either due to lack of proper culture media or built-in-senescence mechanism.
7. Some cells continue to grow after numerous transformations; these are termed as diploid cell strains; these cells also lose the ability to grow after sometimes.
8. Few cells among diploid cell strains will survive; these are termed as heteroploidy cells, because they undergo many chromosomal rearrangements and deletions. These cells will grow indefinitely as long as the medium is replaced, becoming effectively immortal. These survivors are known as cell line.

**Culture medium**

It is the environment provided for the growth of the cells in the laboratory, like those conditions that the cells have been exposed to in vivo. Culture media consists of:

- **Physical media:** a support or matrix.
- **Chemical media:** appropriate nutrients and stromal factors.

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Serum is the most economical, easily available and most widely used culture medium for animal cell culture; fetal calf serum is the preferred one. The major functions of serum as a culture medium are to provide nutrients, hormones, growth factors, attachment and spreading factors, binding proteins, vitamins, minerals, lipids, protease inhibitors and pH buffer.

**Disadvantages of serum**

- Virus, fungi and bacteria may contaminate the serum easily.
- Some enzymes present in serum can convert the cell secretions into toxic compounds.

**Now there are three types of artificial culture media**

- Serum –free culture medium.
- Protein- free culture medium.
- Chemically defined media.

**Types of cell cultures**

**(A) Primary cell culture:**

The maintenance of growth of cells dissociated from the parental tissue in culture medium using suitable glass or plastic containers is called Primary Cell Culture. There are two types of it:

1. Monolayer cultures or adherent cells; cells shown to require the attachment for growth (most cells are anchorage dependent and must be cultured with sterile procedures while attached to a solid or semi-solid substrate). They are usually derived from tissues of organs.
2. Suspension culture; cells which do not require attachment for growth. They are derived from cells of the blood system.

**Advantages in propagation of cells by suspension culture method:**

- The process of propagation is much faster.
- The frequent replacement of the medium is not required.
- Have a short lag period.
- Treatment with trypsin is not required.
- A homogenous suspension of cells is obtained.
- The maintenance of them is easy and bulk production of the cells is easily achieved.

### **(B) Secondary cell cultures or cell line:**

When a primary culture is sub-cultured, it becomes known as secondary culture or cell line. Subculture (or passage; is the transfer of cells from one culture vessel to another culture vessel).

### **There are two types of Cell Line or Cell Strain**

#### **1. Finite cell Lines:**

- Have a limited lifespan.
- They grow in monolayer form.
- Exhibit the property of contact inhibition.
- The growth rate is slow.
- The doubling time is around 24-96 hours.

#### **2. Continuous Cell Lines:**

- Have unlimited life span.
- Exhibit heterogeneity.
- They grow in monolayer or suspension form.
- Absence of contact inhibition.
- The growth rate is rapid; the doubling time is 12-24 hours.

### **Cell Culture Equipment**

#### **Basic Equipment:**

- Cell culture hood (biosafety cabinet).
- Incubator (humid CO<sub>2</sub> incubator recommended).
- Water bath.
- Centrifuge.
- Refrigerator and freezer (-20°C).
- Cell counter (e.g., countess automated cell counter or hemacytometer).
- Microscope.
- Liquid nitrogen (N<sub>2</sub>) freezer or cryo-storage container
- Sterilizer (i.e., autoclave).

#### **Expanded Equipment:**

- Aspiration pump (peristaltic or vacuum).
- pH meter.
- Confocal microscope.
- Flow cytometer.

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**Additional Supplies:**

- Culture vessels (e.g. flasks, Petri dishes, roller bottles, multi-well plates).
- Pipettes.
- Syringes and needles.
- Waste containers.
- Media, sera, and reagents.
- Cells.

**Selecting the Appropriate Cell Line**

- **Species:** non-human and non-primate cell lines usually have fewer biosafety restrictions, but ultimately your experiments will dictate whether to use species specific cultures or not.
- **Functional characteristic:** what is the purpose of your experiments? For example, liver and kidney derived cell lines may be more suitable for toxicity testing.
- **Finite or continuous:** while choosing from finite cell lines may give you more options to express the correct functions, continuous cell lines are often easier to clone and maintain.
- **Normal or transformed:** transformed cell lines usually have an increased growth rate and higher plating efficiency, are continuous, and require less serum, but they have undergone a permanent change in their phenotype through a genetic transformation.
- **Growth conditions and characteristics:** what are your requirements with respect to growth rate, saturation density, cloning efficiency, and the ability to grow in suspension? For example, to express a recombinant protein in high yields, you might want to choose a cell line with a fast growth rate and an ability to grow in suspension.
- **Other criteria:** if you are using a finite cell line, are there sufficient stocks available? Is the cell line well-characterized, or do you have to perform the validation yourself? If you are using an abnormal cell line, do you have an equivalent normal cell line that you can use as a control? Is the cell line stable? If not, how easy is it to clone it and generate sufficient frozen stocks for your experiments?

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## **Morphology of Cells in Culture**

They can be divided into three basic categories based on their shape and appearance (i.e., morphology):

1. Fibroblastic (or fibroblast-like) cells are bipolar or multipolar, have elongated shapes, and grow attached to a substrate.
2. Epithelial-like cells are polygonal with more regular dimensions and grow attached to a substrate in discrete patches.
3. Lymphoblast-like cells are spherical and usually grown in suspension without attaching to a surface.

## **Applications of animal cell culture**

1. Used as substitute hosts to study the pattern of viral infection.
2. Used in manufacture of vaccines, antibodies, hormones, interferon, vitamins, steroids, pharmaceutical drugs...etc.
3. They are good tools for testing the potency of drugs.
4. They served as models to study the metabolism of various substances.
5. They are used in study of the effects of toxins and contaminants.
6. Cancer research, to study uncontrolled cell division in cultures.
7. Cell fusion techniques.

## **Cell Culture Laboratory**

In addition to the safety risks common to most everyday workplaces such as electrical and fire hazards, a cell culture laboratory has specific hazards associated with handling and manipulating human or animal cells and tissues, as well as toxic, corrosive, or mutagenic solvents and reagents. The most common of these hazards are accidental punctures with syringe needles or other contaminated sharps, spills and splashes onto skin and mucous membranes, ingestion through mouth pipetting, and inhalation exposure to infectious aerosols.

Fundamental objective of any biosafety program is to reduce or eliminate exposure of laboratory workers and the outside environment to potentially harmful biological agents. The most important element of safety in a cell culture laboratory is the strict adherence to standard microbiological practices and techniques.

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The following recommendations are simply guidelines for safe laboratory practices, and they should not be interpreted as a complete code of practice:

- Always wear appropriate personal protective equipment. Change gloves when contaminated and dispose of used gloves with other contaminated laboratory waste.
- Wash your hands after working with potentially hazardous materials and before leaving the laboratory.
- Do not eat, drink, smoke, handle contact lenses, apply cosmetics, or store food for human consumption in the laboratory.
- Follow the institutional policies regarding safe handling of sharps (i.e., needles, scalpels, pipettes, and broken glassware).
- Take care to minimize the creation of aerosols and/or splashes.
- Decontaminate all work surfaces before and after your experiments, and immediately after any spill or splash of potentially infectious material with an appropriate disinfectant. Clean laboratory equipment routinely, even if it is not contaminated.
- Decontaminate all potentially infectious materials before disposal.
- Report any incidents that may result in exposure to infectious materials to appropriate personnel (e.g., laboratory supervisor, safety officer).