# ANALYSIS OF MAMMALIAN CELL TYPES

**This protocol is based on the EDVOTEK® protocol "Analysis of Mammalian Cell Types".**

**6 groups of students**

## 1. EXPERIMENT OBJECTIVE

This experiment is designed to introduce students to the study of the microscopic structure of cells (histology). At the end of this experiment, students will identify several major cell lines by examining the morphology. This allows students to connect the concepts of cell structure with its function.

All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

## 2. EXPERIMENT COMPONENTS for 6 groups of students

| COMPONENTS | Store at |
|---|---|
| Ready-to-Stain multispot slides (4 cell types each) | Room temperature |
| Cell fixing agent | Room temperature |
| Eosin stain | Room temperature |
| Methylene blue | Room temperature |
| Mounting medium | Room temperature |
| Slide covers | Room temperature |
| Transfer pipets | Room temperature |

**NOTE:** Store entire experiment at room temperature.

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### 2.1 Requeriments (Not included with this kit)

- Microscopes.
- Forceps.
- Distilled water.
- Gloves.
- Safety goggles.

## 3. BACKGROUND INFORMATION

**CELL MORPHOLOGY**

**Cell morphology** refers to the shape, appearance and structure of a cell. These characteristics are visualized by phase contrast, confocal or electron microscopy.

The morphology of a cell in culture is closely related to the functions of cells within the tissue from which they are derived. The major types of cells that are commonly cultured include **fibroblasts, epithelial cells and lymphoblasts**.

**CELL ATTACHMENT**

**Cell adhesion** plays a critical role in development, wound healing and tumor invasion, as the attachment of cells to the extracellular matrix and to one another is crucial for the maintenance of tissue structure and integrity. Multiprotein complexes called **hemidesmosomes** are responsible for adhesion of epithelial cells to the underlying basement membrane, a layer of fibrous proteins that separates epithelial cells from the underlying connective tissue. Their importance has become apparent in clinical conditions, in which absence or defects of hemidesmosomal proteins result in devastating blistering diseases of the skin.

The lateral connections between adjacent cells are tight and are characterized by the presence of desmosomes and tight junctions. These lateral connections junctions form an impermeable barrier that prevents bacteria from invading the body. **Gap junctions**, formed by connexins, allows for cells to communicate with one another through the transport of molecules between cells.

**MAMMALIAN CELLS**

**Mammalian cells** are cells that are derived or isolated from tissue of a mammal. In this experiment, students are introduced to four mammalian cell types: fibroblasts, epithelial cells, lymphocytes and macrophages. Lymphocytes are found within the blood. Epidermal cells, fibroblasts, and macrophages are found within the tissues.

**FIBROBLASTS**

**Fibroblasts** are present in almost every tissue type (**Figure 1**). Fibroblasts originate from the embryonic mesoderm, as evidenced by the presence of the intermediate filament protein vimentin. This protein, along with actin and tubulin, forms the structural support of the cell. In vivo, fibroblasts exist as single fusiform cells (wide in the middle and tapered at the ends) embedded within connective tissue.

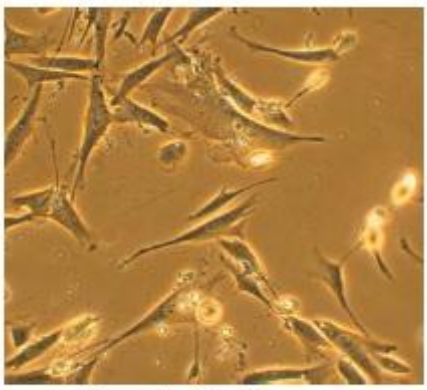


Figure 1: Fibroblasts

Fibroblasts secrete proteins that are important for the formation of the extracellular matrix, including collagen, elastase, fibronectin and laminin. Fibroblasts also play an important role in normal physiological processes such as wound healing by secreting matrix proteins, growth factors and cytokines. Tissue damage stimulates fibroblasts to differentiate into myofibroblasts, which contract to close the wound.

Fibroblasts exhibit **contact inhibition**, a growth control mechanism designed to keep fibroblasts growing into monolayers when in contact with neighboring cells. Fibroblasts also play an important role in many disease states. Overproduction of connective tissue can change the normal morphology of a tissue, resulting in a condition called **fibrosis**. Transformed fibroblasts can also give rise to a type of cancer called **sarcoma**, which comprises tumors from cells of mesenchymal origin (bone, cartilage, fat).

***Human Foreskin Fibroblasts (HS27)***, an extremely well studied cell line, represent fibroblasts in this experiment. In cell culture, these cells exhibit different morphologies depending upon the culture conditions, genotype, and phase of the cell cycle. Most times, fibroblasts maintain their characteristic fusiform morphology. However, in the G2/M phase of the cell cycle, fibroblasts may adopt a rounded shape in preparation for cell division (mitosis and cytokinesis).

**EPITHELIAL CELLS**

Epithelial cells are derived from can be derived from all three germ layers, though true epithelium is considered to be ecctodermal (**Figure 2**). These cells are found as one of three shapes in the body–squamous (flat, scale like cells), cuboidal (height and width are the same) and columnar (cells are taller than they are wide). Epithelial cells develop into sheets or tubes that separate the organism from its environment, allowing for the survival of multicellular organ- isms. These tissues are found throughout the body, including the epidermis, digestive system, reproductive system, and endocrine system.

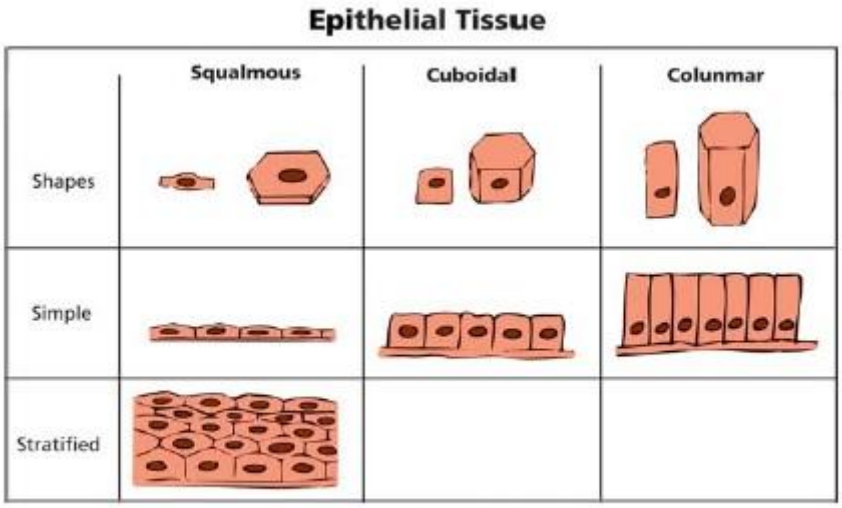



Figure 2: Epithelial Cells

Epithelial tissues are classified as **simple** (stomach, intestine, kidney), stratified (epidermis, esophagus, tongue), or **pseudostratified** (trachea). Simple and pseudostratified epithelia consist of a single layer of cells that rest on a basement membrane. In contrast, **stratified** epithelia consist of two or more layers, with only the dividing layer of cells attached to the basement membrane. Communication between epithelial cells allows the tissues to respond in a coordinated fashion to growth, differentiation, and wound healing. In the transformed state, **keratinocytes** and other epithelial cells give rise to **carcinomas**.

In culture, epithelial cells adhere tightly to the substrate, resulting in flattened cell morphology. The cells will form a contiguous monolayer due to contact inhibition, the process by which monolayers of cells cease to divide and migrate once they come into contact with each other. The intermediate filament protein keratin is also used as a cellular marker for epithelial cells in cell culture.

For this experiment, epithelial cells are represented by human keratinocytes that have been immortalized using (noninfectious) oncogenes of **human papillomavirus (HPV)**. Although these cells are immortalized, they are not transformed, so they retain all the characteristics of primary human keratinocytes.

**LYMPHOCYTES**

Both red and white blood cells originate from a **totipotent cell** found in the bone marrow called a **hematopoietic stem cell**. The program of differentiation from these stem cells appears to depend on both intrinsic and external factors. **Red blood cells** are responsible for delivering oxygen to the body through the circulatory system. **White blood cells**, or **leukocytes**, are responsible for the immune response in mammals.

Leukocytes further differentiate into **lymphocytes**, **granulocytes** and **monocytes**. **Lymphocytes** give rise to **T and B cells**, which are responsible for cellular and humoral immunity, respectively (**Figure 3**). **T lymphocytes** further differentiate into **T helper**, **T suppressor**, and cytotoxic T cells. T helper cells stimulate antibody production and release in B cells.

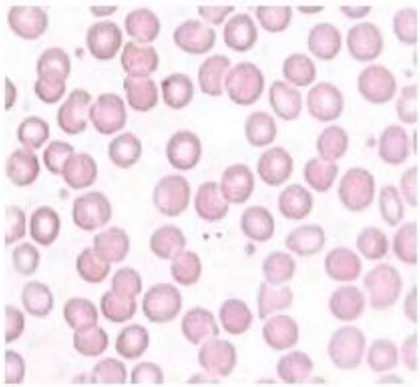


Figure 3: Lymphocytes

Unstimulated T and B cells are very similar in appearance, even when imaged using high-resolution electron microscopy. Both types of cells are small (only marginally bigger than red blood cells). Very little cytoplasm is observable, as the nucleus occupies most of the cell. Lymphocytes exist as rounded cells in vivo as well as in cell culture. Under normal cell culture conditions, lymphocytes do not adhere to the substrate.

**HUT78 Lymphocytes** were chosen to represent non-adherent leukocytes in this experiment. These cells were derived from peripheral blood of a patient with **Sezary syndrome**, a leukemia characterized by a malignant proliferation of helper T cells. These cells have been cultured for 53 years! They are identified by their small size, round shape, and high nucleocytoplasmic ratio. They are useful for studies of the **Human Immunodeficiency Virus (HIV)**, as they are susceptible to infection by the virus.

**MACROPHAGES**

**Macrophages** do not exhibit contact inhibition and are produced through the differentiation of monocytes, a type of white blood cell in mammals (**Figure 4**). They are specialized cells with responsibilities in both the **innate and adaptive immune system**. Macrophages contribute to the innate immune response through phagocytosis of foreign materials (like microbes and fragments of dying cells). In response to pathogens, macrophages secrete cytokines that in turn activate the adaptive immune system. Macrophages are highly motile cells; they move through the body in an amoeboid fashion, which allows them to move to the site of an infection or tissue damage.

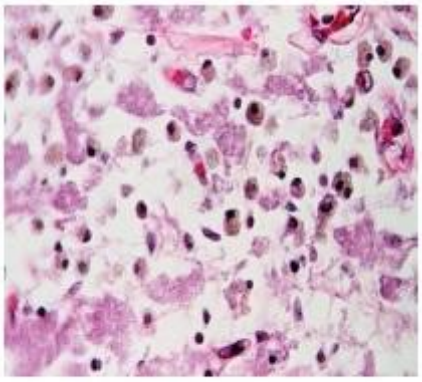


Figure 4: Macrophages

Macrophages are studied in culture for their role in immunity, but also because these cells are specifically targeted by pathogens like tuberculosis and HIV. Both macrophages and monocytes can adhere to the substrate during growth in culture, although not as tightly as epithelial and fibroblasts. This results in a more spherical shaped cell. Like fibroblasts and other mesodermal cell types, monocytes and macrophages can be identified by the presence of vimentin.

In this experiment, ***Mus musculus*** **(mouse) AP388 macrophages** are used as a representative of this cell type. These cells were derived from a lymphoid tumor generated by treatment with methylcholanthrene, a known carcinogen. Although this is a transformed cell line, the cells retain the characteristics of normal macrophages. As with primary macrophages, these cells are active in antibody-dependent cell-mediated cytotoxicity systems and they phagocytize zymosan and latex beads. AP388 express receptors for **immunoglobulin** (Fc) and **complement** (C3), which are required for their function in immunity.

They also express the growth factor **interleukin-1** (IL-1) when stimulated with **lipopolysaccharide** (LPS) or **phorbol myristic acid** (PMA).

## 4. EXPERIMENTAL PROCEDURES

This experiment is designed to introduce students to the study of the microscopic structure of cells (histology). At the end of this experiment, students will identify several major cell lines by examining the morphology. This allows students to connect the concepts of cell structure with its function.

### 4.1 Safety

1. Gloves and safety goggles should be worn at all times as good laboratory practice.

2. NOT PIPETTE WITH THE MOUTH, use appropriate devices.

3. Exercise caution when working with equipment using together heat and mix of reagents.

4. Wash hands with soap and water after working in the laboratory or after using biological reagents and materials.

If you are unsure of something, ASK YOUR INSTRUCTOR

### 4.2 PreLabs preparations

**Notes preparations teacher practice**

The class size, length of classes of practices and equipment availability are factors that must be considered in the planning and implementation of this practice with their students. These guidelines can be adapted to fit your specific circumstances.

**Laboratory notebooks**:

Scientists document everything that happens during an experiment, including experimental conditions, thoughts and observations while conducting the experiment, and, of course, any data collected. Today, you'll be document- ing your experiment in a laboratory notebook or on a separate worksheet.

**Registration laboratory activities**

Students must register in their book practices the activities listed below.

Before starting practice:
- Write a hypothesis that reflects practice.
- Predict the experimental results.

During practice:
- Register (drawing) comments, or photograph the results.

At the end of practice:
- Develop an explanation of the results.
- Determine what could change in practice if you repeat.
- Write a hypothesis reflect this change.

### 4.3 Material that should receive each group

Distribute the following to each student group, or set up a work station for students to share materials.

- 1 Ready to Stain slide
- Fixing reagent
- Staining reagents
- Mounting medium
- 3 transfer pipets
- 1 pair forceps
- Distilled water (50 ml.)
- Kimwipes

## 5. STUDENTS EXPERIMENTAL PROCEDURES

### Staining Procedure

1. Fix slides containing adherent cells by placing two to three drops of fixing agent on each cell spot. Incubate 5 minutes at room temperature.

2. Air dry slides in hood.
3. Add one to two drops of blue stain (methylene blue) to each area of the slide containing the cells.
4. Incubate the slide(s) for 7 minutes at room temperature.
5. Aspirate the methylene blue with a transfer pipet. Do not wash.
6. Add one drop of pink stain (eosin) to the same area. Incubate for 30 seconds at room temperature.
7. Rinse the slide(s) briefly with tap water. Gently tap or "wick" the slides onto a paper towel to remove excess water. Mount the slide(s) by placing one small drop of mounting medium onto the cells, then carefully placing the coverslip(s) on top.
8. View by bright field microscopy.

### Observación microscopic

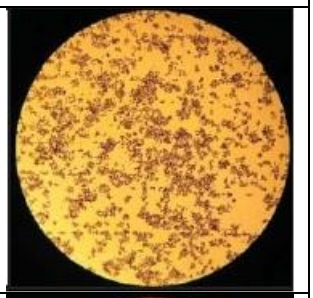
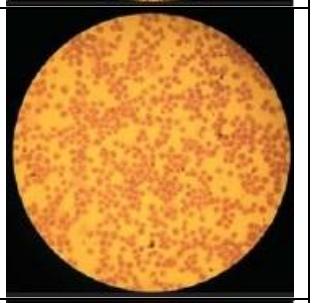
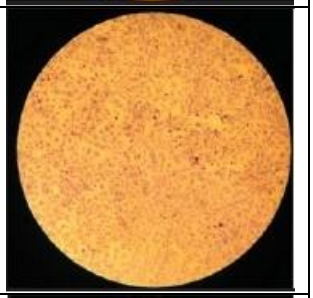
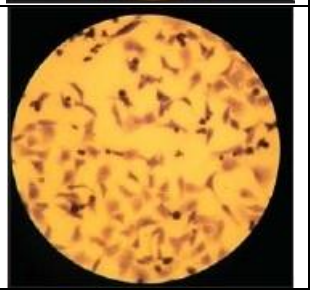
1. While on low power objective (10X or 20X), observe the overall morphology of each cell type, focusing on the center rather than the edge of each slide. Hints: Are cells dense or sparse? Round or polygonal? Regularly or irregularly shaped?
2. Switch to high power objective, which allows you to see more details in the culture.
3. Classify each cell type as fibroblast, keratinocytes, lymphocyte, or macrophage. Note your observations in table below.
4. What is the shape of the nucleus? What is the relative size of the nucleus compared to the cytoplasm? Note your observations in table below.

| Cell Type | Low Power Observation | High Power Observation | Classification |
|---|---|---|---|
| 1 | | | |
| 2 | | | |
| 3 | | | |
| 4 | | | |

## 6. RESULTS AND PRACTICE QUESTIONS

### 6.1 Experimental Results and Analysis

The order of the cells on the slide from frosted end: Macrophages, Lymphocytes, Fibroblasts, Keratinocytes.

| Cell type | Low Power Observations | High Power Observation | Classification | Photo |
|---|---|---|---|---|
| 1 | Clusters of round cells | Large in size, mononuclear cells | **Macrophages** |  |
| 2 | Cluster of circular cells | Nearly colorless, non granular cells | **Lymphocytes** |  |
| 3 | Polygonal and irregularly shaped cells | Flat, elongated cells | **Fibroblasts** |  |
| 4 | Relatively symmetrical, crescent shaped morphology | Dark nucleus in the center | **Keratinocytes** |  |

## 6.2 Study Questions

Answer the following questions in the lab notebook:

1. How does the morphology of each cell type relate to its function?
2. Give an example of external stratified epithelium, and internal stratified epithelium.
3. Which cell types are found within the blood? Which cell types are found within the tissues?
4. Which cell types are the largest? The smallest? What would be the advantage of a smaller cell?
5. Which cells are the most adherent (flattest)? The least adherent? What is the advantage of each?