FUNDAMENTAL TISSUES AND MITOSIS

Medical School Histology
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Part I
Types of microscopy, tissue preparation, and staining
Objectives

Part I

• Operate the “virtual microscope”
• Describe steps for tissue processing and discuss different types of microscopy
• Interpret cytological significance of differential staining by hematoxylin and eosin (H&E)
• Recognize and identify cells in microscopic tissue preparations
• Understand that morphology reflects the function of cells
• Identify stages of mitosis and cellular structures associated with mitosis

From: Douglas P. Dohrman and TAMHSC Faculty 2012 Structure and Function of Human Organ Systems, Histology Laboratory Manual
The Microscope

Light (bright field) used with stained specimens
Kohler illumination

1. Focus specimen
2. Close field diaphragm
3. Focus condenser
4. Center condenser
5. Open field diaphragm
Types of Microscopy

- **Light (bright field)**
  - Most common, used to observe stained sections.

- **Phase Contrast**
  - Used to observe living cells, cells appear as black and white image

- **Nomarski (differential interference)**
  - Similar to phase contrast, but greater resolution of 3D appearance and optical sectioning

- **Dark Field**
  - Illuminates cells and tissues against dark background to allow for contrast

- **Transmission Electron Microscopy (TEM)**
  - Magnifies image to allow visualization of intracellular structures

- **Scanning Electron Microscopy (SEM)**
  - Scans cell surfaces at great magnifications providing highly resolved surface details
• **Bright Field**
  - Stained dead cells

• **Phase Contrast**
  - Live unstained cells

• **Nomarski**
  - Differential interference contrast

• **Dark Field**
  - Dark, contrasting background
Types of Electron Micrographs

Conventional TEM, SEM, carbon replica TEM
Types of Electron Micrographs
Types of Electron Micrographs

- Conventional TEM, SEM
- Carbon replica TEM
Transmission Electron Microscopy (TEM)
Scanning Electron Microscopy (SEM)

ELASTIC ARTERY

SEM

KIDNEY-RENAL CORPUSCLE

SEM

EC = Endothelial Cells
LU = Lumen

CS = Capsular Space
PL = Parietal Layer Bowman's Capsule
PO = Podocytes
Arrows = Podocyte Process
Transmission Electron Microscopy (TEM)

Comparing Magnifications

Compare the magnification by comparing sizes of:
- membranes
- ribosomes
- mitochondria
Compare sizes
• membranes
• ribosomes
• mitochondria
Tissue Preparation

1. **Fixation** – Chemically prevents autolysis of cells and preserves morphology
2. **Dehydration** – organic solvents
3. **Embedding** – paraffin or plastic used to stiffen tissue
4. **Sectioning** – cutting thin sections (1-10 microns thick)
5. **Staining** – different stains react with different biochemical components of cells
6. **Mounting** – preserve section
7. **Histological viewing** – using microscope
Sample Preparation

1. Fixation

2. Embedding
   A. Paraffin
   B. Plastic

3. Sectioning
   A. 0.5 μm for Light Microscopy
   B. 60-80 nm for Electron Microscopy
movement of microtome arm
specimen embedded in wax or resin
steel blade
ribbon of thin sections
ribbon of sections on glass slide, stained and mounted under a cover slip

examination with light microscope

eyepiece
objective lens
condenser
Differential Staining Properties

Hematoxylin and Eosin (H&E) are the most widely used stains

- **Hematoxylin**
  - Blue-black dye with basic pH, preferentially binds acid molecules
  - Nuclear DNA and cytoplasmic ribosomes darkly stain with hematoxylin based on density of material

- **Eosin**
  - Red dye with acidic pH, preferentially binds basic molecules
  - Proteins (amines) stain with eosin based on density of material

- Dark blue nuclei of lymphocytes
- Red cytoplasm of smooth muscle cells
Hematoxylin and Eosin (H&E) Stain

• Slide 32: Kidney (H&E)
Periodic Acid-Schiff (PAS)

• Used to observe saccharides (hot pink of brush border and basement membrane)

• Slide 33: Kidney (PAS)
Staining

1. Light Microscopy
   A. Hematoxylin and Eosin (H&E)
   B. Periodic Acid/Shiff (PAS)
   C. Toluidine Blue

2. Electron Microscopy (TEM)
   A. Osmium
   B. Lead Citrate
Staining

1. **Light Microscopy**
   A. Hematoxylin and Eosin (H&E)
   B. Periodic Acid/Shiff (PAS)
   C. Toluidine Blue

Color provides clues

Shape
Size
Intensity of staining
Staining

1. Light Microscopy
   Hematoxylin and Eosin (H&E)

2. Electron Microscopy (TEM)
   Lead citrate
Artifacts

- Artifacts are man-made distortions and aberrations of tissue structure
- Artifacts are fairly common, beware!
- Slide 32: Kidney
Identifying Cells

- Cells < Tissues < Organ < Organ System < Human

- Somatic vs. Germ Cells

- Morphology accomplishes specific function

- Distinct features of cell
  - Nucleus: shape is indicative of cell morphology
  - Cytoplasm: Contains various organelles such as RER, Golgi, and mitochondria
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Part II
Images of light and electron microscopy
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Part II

• Recognize and identify cells in microscopic tissue preparations
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Laboratory Experience

• Primary purpose of this lab is to gain a general impression of similarities and differences in cells and tissues and what can be inferred from that.

• Do not be too concerned about detailed structure, but rather try to gain an overall appreciation for histology.

USE YOUR ATLAS!
Slide 29: Skin

Epithelial skin cells

Collagen fibers
Slide 29: Skin

Epithelial skin cells

Collagen fibers
Slide 51: Tongue (cross section)

Skeletal muscle

Serous glands

Adipocytes
Slide 51: Tongue (cross section)

- Serous glands
- Adipocytes
- Skeletal muscle
Slide 32: Kidney

- Proximal convoluted tubules with microvilli lining lumen
Slide 75: Thyroid

- Spherical epithelial follicles containing thyroglobulin
- Simple cuboidal epithelium
- Extracellular collagen fibrils
- Blood vessel with red blood cells
- Simple squamous epithelium lining blood vessel
Slide 70: Pancreas

Zymogen granules

Pancreatic acinus

Glandular cell with euchromatic nucleus and basophilic base
Endoplasmic Reticulum

Granular ER

Nuclear pore

Nucleus

Nucleolus

Nuclear envelope

PANCREATIC ACINAR CELL
Granular vs. Agranular Endoplasmic Reticulum

Granular endoplasmic reticulum

Agranular endoplasmic reticulum
Plasma Membrane

What is the structure of the plasma membrane?
Plasma Membrane

FREEZE-FRACTURE OF CELL MEMBRANE

- E-FACE (OUTER)
- P-FACE (INNER)

FREEZE-FRACTURE OF CELL

- N - nucleus
- np - nuclear pore
- ne - nuclear envelope
- V - vacuole
- G - golgi complex
Plasma Membrane

Identify pinocytotic vesicles
Mitochondria

Identify mitochondria
Mitochondria

What is the structure and function of mitochondria?
Mitochondria & Microbodies

- Could mitochondria and microbodies be confused morphologically?

- What is the structure and function of microbodies?
Golgi complex

Observe the ultrastructure of the Golgi complex
Digestion and Storage Vesicles

- Lipid droplets
- Secretory granules
- Lysosomes
Digestion and Storage Vesicles

- Lipofuscin granules
- Microbodies
- Glycogen
Cytoskeleton

Microtubules

Axonemes encased in membrane

Centrioles
Cytoskeleton

• The microvilli in this micrograph contain what type of cytoskeletal element?
  • Hint: What is supporting the microvilli?
Clinical Correlation

• Cell death can occur due to apoptosis or necrosis

• **Apoptosis**
  • Programmed cell death

• **Necrosis**
  • Accidental cell death resulting from pathological condition

![Necrotic neuron vs Apoptotic neuron](image)
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Part III
Images of mitosis
Objectives

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Part III

- Identify stages of mitosis and cellular structures associated with mitosis

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Mitosis

• Mitosis is cellular multiplication
• This is the means by which organisms grow
• Cancer is essentially uncontrolled mitosis
• We will look at onion root tip and fish egg cells to identify the stages of mitosis
Slide 1: Onion root tip
Interphase

Interphase nucleus
Slide 1: Onion root tip
Prophase
Slide 1: Onion root tip
Metaphase

Spindle fibers
Slide 1: Onion root tip
Anaphase

Centrosomal area
Slide 1: Onion root tip
Telophase
Slide 2: Fish egg mitosis

Interphase

Interphase nucleus
Slide 2: Fish egg mitosis
Prophase
Slide 2: Fish egg mitosis
Metaphase

Spindle fibers
Slide 2: Fish egg mitosis
Anaphase
Slide 2: Fish egg mitosis
Telophase
Describe the composition of the nucleolus and the nucleus.

What happens to the nucleolus and nuclear membrane (envelope) during mitosis?
What is the function of centrioles?
Clinical Correlation

- Malignant vs. benign tumors
  - Increased mitotic figures and abnormal mitoses helps distinguish
- Anti-mitotic drugs are used in cancer chemotherapy
  - Ex: Taxol
  - These drugs also effect normal cells with high proliferation rates causing undesirable consequences

http://ttc.nci.nih.gov/about/success_taxol.php
Study Questions

1. Does a highly active cell contain a euchromatic or heterochromatic nucleus?

2. Would the cytoplasm of a cell that contains large quantities of protein stain eosinophilic or basophilic?

3. Is a rounded (spherical) nucleus indicative of a squamous, cuboidal or columnar cell?

4. Does hematoxylin preferentially stain chromatin or collagen? (2-3 sentences)

5. Are polysaccharides and mucopolysaccharides best stained with eosin, Sudan black, hematoxylin, or periodic acid – Schiff?

6. At which stage of mitosis do sister chromatids separate from each other?

7. During which phase of interphase does the first “checkpoint” occur? What is the significance of the p53 protein?

From: Douglas P. Dohrman and TAMHSC Faculty 2012 Structure and Function of Human Organ Systems, Histology Laboratory Manual
Use your atlas!
Many illustrations in these VIBS Histology YouTube videos were modified from the following books and sources: Many thanks to original sources!

- Douglas P. Dohrman and TAMHSC Faculty 2012 Structure and Function of Human Organ Systems, Histology Laboratory Manual - Slide selections were largely based on this manual for first year medical students at TAMHSC
The End!