HISTOLOGY Lecture 1
Introduction, techniques, epithelia and skin
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Learning outcomes

After this module component, you should be able to:

- Describe the microscopic anatomy of different tissues and organs and relate this to their function in the human body
- Recognize the microscopic structures of different human tissues and organs by looking at slides and/or pictures
Scope of the histology component

- Tissue study methods
- BASIC TISSUES
  - *The epithelia; skin and glands*
  - *Connective tissues and bone*
  - *Muscle*
  - *Nervous tissues:*
- Blood, lymphatics and immune system
- Gastro-intestinal system
- Respiratory system
- Urinary system
- Endocrine glands
- Reproductive system
Introduction

- the body is formed of different levels of organization, with increasing levels of complexity and each of which plays important roles in the physiological homeostasis of the body.

**LEVELS OF ORGANIZATION**

- Cells
- Tissues
- Organs
- Organ Systems
- Organism
Introduction

"Histology“:
- "Histos“ for tissue
- and "-logos" = the study of

Histology as a subject includes far more than just the study of tissues, but includes understanding of the structure and function of cells, tissues, organs and organ systems.
1: Tissue study methods

- Tissues are fixed in different ways to demonstrate their structural and functional characteristics.
- It is necessary to preserve the tissues (fixation) and embed them in a supporting medium (such as paraffin wax or resins) prior to sectioning and staining.
- To enable tissue recognition and diagnosis of different conditions.
1: Tissue study methods - Fixation

- tissues degenerate due to bacterial destruction and autolysis
- preserve tissues and prevent structural change or breakdown of the components of the tissues
- Fixative preserves the tissues as close as possible to the living state.
1: Tissue study methods - Fixation

Types of fixatives:

- aldehyde based fixative - formalin (formaldehyde) or in combination with other chemicals - i.e. Bouins - most commonly used general fixative

- Alcohol fixation - usually the fixative used to prepare blood smears

- Freeze tissue (in liquid nitrogen)

- physiological buffers: Glutaraldehyde, Osmium tetroxide (OsO₄) – EM
1: Tissue study methods - Fixation

- Rules for good fixation:
  1) use small pieces of tissue
  2) use large volume of fix - 10x volume of tissue.
  3) get tissue into fix FAST - keep cold (on ice) and wet (in buffer) during dissection - always wash off excess blood with buffer before fix
1: Tissue study methods - Embedding

- The most commonly used: paraffin wax, with a melting point of about 56°C
- Prior to embedding: *Dehydration* is achieved using an ascending series of alcohols (70%, 95%, 100%)
- This is followed by *tissue immersion* in a wax solvent such as xylene or chloroform
- The tissue is then transferred to *molten paraffin wax* (in an embedding oven) for a couple of hours then a *wax block* if formed
1: Tissue study methods - Microtomy

- Microtome

- Sectioning of the embedded tissue into smaller thickness to allow staining and mounting
  - 7 micron sections for LM - placed on glass slides
  - 70 millimicrons - placed on metal grids for EM
1: Tissue study methods - Staining

- must be rehydrated first
- Tissues stain with acid or base stains
  - **ACIDOPHILIC** (stain basic component - i.e. “Normal” cytoplasm)
  - **BASOPHILIC** (stain acid component - i.e. Nuclei or RER in secretory cells)

- After staining, the specimen is mounted on to a slide (glass or metal plates) for viewing under different microscopes – Light or Electron (Transmission, scanning) microscope
1: Tissue study methods - Staining

Types of stains:

- Hematoxylin & Eosin (H & E) - most common stain - good for general structure
  - nuclei = blue (basophilic) → Hematoxylin
  - cytoplasm = pink (acidophilic) → Eosin
1: Tissue study methods - Staining

Types of stains:

- Connective tissue stains - both employ a nuclear, cytoplasmic, and a third stain specific for matrix
  - Masson's trichrome
  - Mallory's triple C.T. stain

Masson’s Trichrome: blue is collagen
1: Tissue study methods - Staining

Types of stains:

- **Silver Impregnation**
  - Specificity provided by what silver is complexed to and pH of staining solution
  - Used to trace nerves, stain golgi, reticular fibers

Silver staining for reticular fibers
1: Tissue study methods - Staining

Types of stains:

- **PAS** (Periodic Acid Schiff's)
  - Schiff reagent a stain called Basic Fuchsin = specific stain for carbohydrates = PAS stain

PAS stain of epithelial basement membrane, pink are neutral sugars
1: Tissue study methods - Staining

Types of stains:

- **Giemsa stain**: Commonly used for blood smear staining
- **Histochemical staining**: for enzymes
- **Immunofluorescence techniques**: for antigen detection with antibodies
- **Toluidine blue, somium tetraoxide, oil red, vital stains, orcein etc**

Giemsa stain of a lymphocyte in peripheral blood
1. For the next session: Epithelia, glands and Skin

2. Review:
   - definition of histology
   - tissue preparation stages
   - types of stains
2: The Epithelia and glands
Concept of Epithelium

- a tissue composed of cells, tightly-bound to each other, with no intercellular connective tissue

- Lines body surfaces, cavities and tubes

- Adapt to different functions depending on location e.g. protection, absorption, secretion etc to maintain physiological homeostasis

- Interfaces between biological compartments
Characteristics of an epithelium

- a basement membrane (glycoprotein ground substance)
- Avascular
- develops in the embryo from all the three germ layers (Ectoderm, Mesoderm, Endoderm) e.g.
  - Skin – Ectoderm
  - Peritoneum – Mesoderm
  - Intestinal mucosa – Endoderm tissue
Functions of an epithelium

- 2 main functions
  - **Protection**: mechanical damage, loss of fluids (desiccation) – waterproofing, invasion of foreign bodies or chemicals
  - **Metabolic**: Exchange of metabolites either absorption or excretion, secretion, sensory reception
Structure of an epithelium

- cells are polarized with:
  - Apical surface: free surface interfacing with external environment and may have features such as microvilli, stereocilia, cilia or flagella.
  - Lateral surfaces: have "junctional complexes" including:
    - **tight junctions** ((impermeable)
    - **adhering junctions** (desmosomes - promoting adhesion)
    - **communicating junctions** (gap junctions or nexuses - allow the exchange of nutrients, ions, signals between adjacent cells)
  - Basal surfaces
### Junctional Complexes

<table>
<thead>
<tr>
<th>name</th>
<th>function</th>
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<tbody>
<tr>
<td>tight junction</td>
<td>seals neighboring cells together in an epithelial sheet to prevent leakage of molecules between them</td>
</tr>
<tr>
<td>adherens junction</td>
<td>joins an actin bundle in one cell to a similar bundle in a neighboring cell</td>
</tr>
<tr>
<td>desmosome</td>
<td>joins the intermediate filaments in one cell to those in a neighbor</td>
</tr>
<tr>
<td>gap junction</td>
<td>forms channels that allow small water-soluble molecules, including ions, to pass from cell to cell</td>
</tr>
<tr>
<td>hemidesmosome</td>
<td>anchors intermediate filaments in a cell to the basal lamina</td>
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*Figure 20-22 Essential Cell Biology 3/e (© Garland Science 2010)*
Tight Junctions (Zonula Occludens) by TEM
Tight Junction diagram

Figure 20-23 Essential Cell Biology 3/e (© Garland Science 2010)
Gap Junction

2 nM gap between membranes

Open pores for ions, molecules, etc

Connexin molecules
TEM (colorized) of Adherens Junctions
Desmosome Structure

- Keratin filaments anchored to cytoplasmic plaque
- Cytoplasmic plaque made of intracellular anchor proteins
- Interacting plasma membranes
- Intercellular space
- Cadherin family proteins

Figure 20-27a Essential Cell Biology 3/e © Garland Science 2010
Figure 20-27b Essential Cell Biology 3/e © Garland Science 2010
Summary of the maintenance of cell shape by junctions and cytoskeletal elements
Morphological classification

- 3 main characteristics:
  - Number of cells: Simple or Stratified
  - Shape of component cells: squamous (flattened); cuboidal (more square or cube-like); columnar (tall and thin); transitional
  - Presence of surface modifications: cilia, keratin, microvilli
Describe this epithelium
Simple epithelia

- On absorptive, excretory or secretory surfaces with no stress forces
  - **Squamous**: allow for easy diffusion/passive transport of substances: alveoli, peritoneum, pleura, blood vessels
  - **Cuboidal (more square or cube-like)**: usu. in small ducts and tubules with excretory, secretory or absorptive function: collecting tubules, salivary glands, pancreas, the bile ductules of the liver, or the cells lining thyroid follicles;
  - **Columnnar**: usu. on highly absorptive surfaces e.g. small intestine; may have microvilli at the apical surface
Simple Squamous Epithelium
Total Preparation - Mesentery
N=nucleus
Simple Squamous – Endothelium (En)
Capillary (endothelial cell) by TEM
Simple Cuboidal Epithelium
Kidney, urinary tubules
Simple Cuboidal Epithelium (longitudinal section)
Simple Cuboidal (cross section)
Simple Cuboidal by TEM
Simple columnar
Simple Columnar with Goblet Cells
Simple Columnar (cross section) with Goblet Cells (arrow)
Goblet Cell with PAS stain (red)
Simple Columnar mucus secreting
Pseudostratified Ciliated Epithelium with Goblet Cells
Pseudostratified Epithelium with Cilia and Goblet Cells
(Respiratory Epithelium)
Stratified epithelia

- Primarily protective
- Surface cells define the classification
- Basal cells undergo regular mitosis to replace the top layers (rate of mitosis according to rate of surface loss)

Common types:

- **Squamous**: oral cavity, pharynx, esophagus, anal skin, vagina, epidermis of the skin
- **Cuboidal**: usu. 2-3 layers; in large exocrine glands e.g. salivary glands, pancreas and sweat glands
- **Transitional** epithelium: highly specialized urothelium;
Stratified Squamous Epithelium
Tongue
Stratified Squamous non-Keratinized Epithelium
Stratified Squamous Keratinized (thin)
Stratified Squamous Epithelium with Keratin
Thick skin. Osmium staining
Sweat gland with double layered duct
Transitional Epithelium
Transitional Epithelium with rounded binucleated surface cells
Glandular Epithelium

- Secretion is one of the epithelial functions
- Glands are invaginations of epithelial surfaces – embryonic proliferation into the underlying connective tissue

Types:
- Exocrine – secrete to free surface
- Endocrine – ductless, secrete directly into blood stream
A: Exocrine glands

- 2 major differentiating properties
  - Morphology:
    - Unicellular e.g. goblet cells – mucin secretion
    - Multicellular: with a secretory duct and a secretory unit
      - simple (with single un-branched duct) or compound (with branched ducts)
  - Means of discharge:
    - Merocrine (eccrine): by exocytosis
    - Apocrine: free membrane-bound vesicles released at the apical end e.g. milk secretion and some sweat glands - axilla
    - Holocrine: whole cells with their contents e.g. sebaceous glands
Types of secretory units

- tubular (e.g. intestinal crypts of Lieberkuhn)
- coiled tubular (e.g. sweat glands)
- branched tubular (e.g. Brunner’s glands of the duodenum)
- alveolar
- acinar (e.g. exocrine pancreas)
Serous Gland with ducts (arrows)
Serous secretory unit (high magnification)
Sweat gland with double layered duct
Sebaceous glands associated with hair follicles
Mucous Gland with duct (arrow)
B: Endocrine glands

- Ductless and surrounded by a rich network of blood capillaries
- Secretory cells arranged in cords and clumps
- Secrete hormones

- The **protein secreting cells** are typically characterized by well-developed RER (rough endoplasmic reticulum), Golgi bodies and membrane-bound secretory granules.

- The **steroid-secreting cells** (e.g. in the testis, ovary, suprarenal cortex) are characterized by well-developed SER (smooth endoplasmic reticulum) and abundant lipid droplets.
Break

In the next 15 minutes
Review:
- different epithelia
- types of glands
3: The Skin
The Skin

- The largest organ in the body ~ 16% of body weight
- The first line of defense (immunity)
- Involved in metabolism
- Composed of two main layers: epidermis and dermis of varying thickness
Functions of the skin

• **Protection**
  - Physical, Temperature, Chemical protection
  - Camouflage in many animals
  - Anti-bacterial secretions

• Prevents evaporation and dehydration

• Body temperature regulation (insulation, sweating, hair)

• Protection from UV light (pigment)
Functions cntd..

• Senses of touch, pain, temperature
• Grip (fingerprints, nails, claws)
• Synthesis of vitamin-D3
• Excretion (water, salts)
• Scent production
• Sexual attraction (visual)
Detailed structure of the skin
The epidermis

- **Epidermis** (keratinocytes most common cell)
- Stratified squamous epithelium
- Layers:
  - *Stratum corneum*: cell remnants, keratin
  - *Stratum lucidum* (thick skin only)
  - *Stratum granulosum*
  - *Stratum spinosum*
  - *Stratum germinativum* (= s. basale): germinal layer
Epidermis

- 4 cell types, with different embryologic origins, are distinguished in the epidermis:
  - Keratinocytes (keratin production)
  - Melanocytes (pigment production)
  - Langerhans cells (immune system)
  - Merkel cells (diffuse neuroendocrine system).
The dermis

- **Papillary Layer** (dermal ridges = fingerprints)
  - Superficial
  - Loose connective tissue
- **Reticular layer**
  - Primarily dense irregular CT (esp. reticular layer)
  - Collagen fibers are found throughout dermis (make skin resilient)

Dermis has rich blood & sensory nerve supply
The Dermis

Papillary Dermis

Reticular Dermis
Skin changes

- With age, collagen and elastin fibers of the dermis break down, producing wrinkles
- Exacerbated by sun exposure
- Wrinkles usually form perpendicular to muscles below skin.
Skin color

- **MELANOCYTES**, produce the pigment *melanin*
- Skin color variation due to size, shape, and activity of melanocytes
- Activity regulated by sun exposure, hormones, genetics
- Protects cells’ DNA from UV damage
- Prevents destruction of folic acid (folate vitamin)
- Limits vit-D3 synthesis
Thin Skin with Melanocytes
Meissner’s Corpuscle within a dermal papilla
Meissner’s Corpuscle
Skin appendages

- **Hairs:**
  - produced by hair follicles
  - Hair bulb: terminal end of follicle with cells in mitosis
  - Arrector pili muscle

- **Sebaceous glands:** sebum offers a waterproof layer; with or without hairs

- **Sweat glands:** merocrine (cholinergic) and apocrine (adrenergic)

- **Nails**
Sweat Glands (Sg) and Ducts (arrow)
Sebaceous glands associated with hair follicles
Germinal matrix of a hair follicle
1. In the next session: Connective tissues, muscles and bone
2. Review:
   - Functions of the skin
   - Basic structure of the skin