ARKAN
ACADEMY

HISTOLOGY

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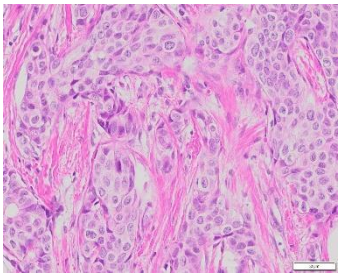
Study smarter, not harder!

Introduction to Histology

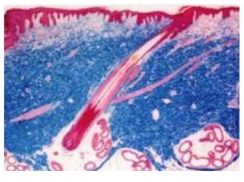
- ***Histology:*** The study of the tissues of the body and how these tissues are arranged to constitute organs
- ***Tissue:*** A group cells surrounded and impeded in ECM
  - *ECM (Extracellular matrix):* solution between the cells, containing water, glycoproteins, electrolytes
- For a tissue to be studied, it must undergo "Tissue Processing", which include:
  - ***Fixation:*** Small pieces of tissue are placed in solutions of chemicals that ***cross-link*** proteins and ***inactivate degradative enzymes***, which ***preserves*** cell and tissue structure
    - ✓ Most popular fixative is *formalin*
  - ***Dehydration:*** The tissue is transferred through a series of increasingly concentrated *alcohol* solutions, ending in 100%, which ***removes all water***
  - ***Clearing:*** ***alcohol is removed*** in an *organic solvent* in which both alcohol and paraffin are miscible
  - ***Infiltration:*** the tissue is placed in *melted paraffin* until it becomes completely infiltrated with it
  - ***Embedding:*** the tissue is placed in a ***small mold*** with *melted paraffin* and allowed to ***harden***
  - ***Trimming:*** the paraffin block is trimmed to expose the tissue for ***sectioning*** (slicing) on a microtome
    - ✓ The perfect thickness of the section is 7-10 μm
  - ***Staining:*** adding a stain (pigment) to ***enhance the contrast*** of the image
    - ✓ Most cells and extracellular material are completely colorless

- Stains are classified into general and special stains
- **General Stains:** Include dyes forming electrostatic interactions with ionizable molecules of the tissues
  - ***Basic dyes:*** include *Hematoxylin*, *toluidine blue*, *alcian blue*, and *methylene blue*
    - ✓ Stain *negatively charged structures* (***anionic***, ***acidic***, ***basophilic***) such as **nucleic acids** (DNA, RNA) & **GAGs** (glycosaminoglycans) with a **blueish color**

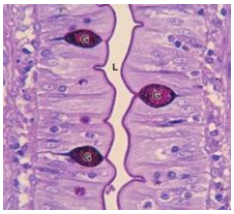


  - ***Acid dyes:*** include *eosin*, *orange g*, and *acid fuchsin*
    - ✓ Stain *positively charged structures* (***cationic***, ***basic***, ***acidophilic***) such as **proteins** with many ionized amino groups, **mitochondria**, **secretory granules**, **collagen** and **cytoplasm** with a **pinkish color**

- **Special stains:** Bind specifically to certain chemical groups and structures
  - ***Trichrome stains:*** allow greater distinctions among various extracellular tissue components such as Masson trichrome
    - ✓ Stain the *connective tissue (blue)*, *cytoplasm (pink)*, *nuclei (dark brown)*

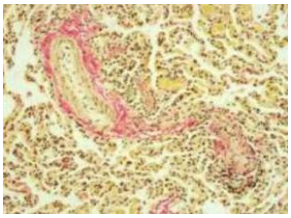


  - ***PAS (periodic acid-Schiff)*** utilizes the hexose rings of ***polysaccharides*** and other ***carbohydrate***-rich tissue structures and stains them distinctly *purple or magenta*



  - ***Sudan black:*** ***lipid-soluble dyes*** satin lipids; **avoiding the processing** steps that remove lipids, such as treatment with heat and organic solvents

- ***Van Gieson method:*** stain *collagen (pink)*, *muscle (yellow)*



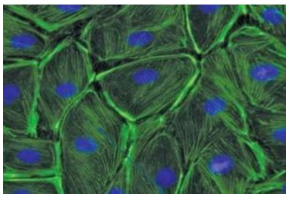
- ***Metal impregnation:*** less common methods, using solutions of ***silver salts*** to visual certain ECM fibers, specific cellular elements in *nervous tissue*
- ***Immunostaining:*** use ***antibodies*** that bind *specific* molecules inside the cell which include immunofluorescence and immunohistochemistry

Microscopes

- Microscopes are instruments that are used to observe small molecules
  - They differ in their quality according to their resolving power depending on the quality of the ***objective lens***
  - ***Resolving power:*** The *smallest distance* between 2 structures to be seen as *separated* objects
  - It determines the ***quality***, clarity and richness of detail
    - ✓ As the microscope distinguish smaller particles, higher resolving power, higher quality

- **Light Microscope:** Uses *light (such as visible or UV light)*
  - Maximum resolution *0.2 μm* (Objects smaller than 0.2 μm such as a single ribosome or cytoplasmic microfilament cannot be distinguished)
  - Has 2 main lens, ocular and objective lens, where its magnification power equals the ***multiplication of the 2-lens power*** (Permit magnification of *1000- 1500 time*)

- Types of light microscope:
  - ***Bright-field microscopy***
    - ✓ Stained tissue is examined with ***ordinary light*** passing through the preparation
    - ✓ Includes an optical system and mechanisms to move and focus the specimen
    - ✓ The *condenser* ***collects and focuses*** a cone of light that illuminates the tissue slide on the stage
    - ✓ *Objective lenses* ***enlarge and project*** the illuminated image x4, x10
    - ✓ *Two eyepieces or oculars* ***magnify*** this image another x10 and project it to the viewer, yielding a total magnification of x40, x100, or x400
  - ***Fluorescence microscopy***



    - ✓ Tissue sections are irradiated with ***Ultraviolet (UV) light*** and the emission is in the visible portion of the spectrum
    - ✓ The fluorescent substances appear bright on a dark background
    - ✓ It has a source of UV or other light and filters that select rays of different wavelengths emitted by the substances to be visualized
    - ✓ It can use an ***Immune-Fluorescent*** staining which is **more specific** (using ***antibodies***)
  - ***Confocal microscopy***
  - ***Polarizing microscopy***

- ***Phase-contrast microscopy***
  - ✓ Study ***unstained*** cells and tissue sections (***colorless***; similar optical densities)
  - ✓ Uses a lens system that produces visible images from ***transparent objects*** and can be used with ***living, cultured cells***
  - ✓ Is based on the principle that light ***changes its speed*** when passing through cellular and extracellular structures with different ***refractive*** indices (appear lighter or darker relation to each other)

- **Electron Microscope:** uses a *beam of electrons*
  - Images results from the refraction and absorption of the electron beam so appears ***white & black***
  - For the light microscope we put the sample on a ***glass slide***, but for the electron microscope we put it on a ***metal grid***

- Types of electron microscope:
  - *Transmission electron microscope (TEM)*
    - ✓ Produces ***2D images*** where the electrons ***penetrate*** the cell and can produce images for the ***internal structures and organelles***
  - *Scanning electron microscope (SEM)*
    - ✓ Produces ***3D images*** where the electrons ***can't penetrate*** the surface producing images for the ***cell surface***

Past Papers

1. *One of the following parts of bright-field microscope focuses light on the object to be studied:*
   - A. Focusing-piece
   - B. Objective lens
   - C. Beamsplitter
   - D. Ocular lens
   - E. Condenser

2. *You are a researcher in nanotechnology interested to understand the ultrastructural distribution of a potential biomaterial used for as an intracellular drug. The most microscope useful to you is*
   - A. Confocal microscope
   - B. Fluorescent microscope using phalloidin
   - C. TEM
   - D. Polarizing microscope
   - E. SEM

Answers
1. E
2. C

3. *Which of the following is a term used to describe the shortest distance between two points that can still be distinguished as separate entities*

   A. Contrast
   B. Resolution
   C. Magnification
   D. Wavelength
   E. Focus

4. *The step of sample preparation that precedes the clearing step with an organic solvent is?*

   A. Fixation
   B. Embedding
   C. Dehydration
   D. Staining
   E. Infiltration

5. *Which of the following stains depends mainly on the cationic and anionic properties of the sample:*

   A. PAS
   B. Immunohistochemistry
   C. Metal impregnation technique
   D. H & E

6. *The resolution of the light microscope depends mainly on:*

   A. Ocular lens
   B. Condenser
   C. Objective lens
   D. Specimen slide

7. *What is the most commonly used chemical for removing water from biological samples (dehydration):*

   A. Xyline
   B. Paraformaldehyde
   C. Alcohol
   D. We can air-dry samples instead
   E. None of the above

8. *What is the most suitable microscope(s) to visualize the following: cultured cells, immunohistochemically stained sections, and H&E-stained sections, respectively:*

   A. Bright-field microscope for all of them
   B. Bright-field microscope, phase-contrast microscope, and bright-field microscope
   C. Phase-contrast microscope, bright-field microscope, and bright-field microscope
   D. Bright-field microscope, immunofluorescence microscope, and TEM
   E. Phase-contrast microscope, immunofluorescence microscope, and bright-field microscope

Answers
3. B
4. C
5. D
6. C
7. C
8. E

9. *Your colleague is planning to found the abundance of myoepithelial cells in parotid glands by staining (as precise and easy as possible), what do you advise her/him to do:*

   A. H&E stain
   B. TEM
   C. PAS stain
   D. Immunohistochemistry or immunofluorescence staining
   E. Any of the above

10. *During the preparation of a routine H&E slide, what step occurs after the tissue is fixation?*

   A. Clearing
   B. Embedding in paraffin
   C. Slicing
   D. Dehydration

Answers
9. D
10. D

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