faculty of medicine - JU2015

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First lecture

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recommended book : janqueira 13th or 14th edition

Histology

Introduction

- **Histology**: the science which deals with tissues.

- Histology is the microscopic Anatomy (study the same structures: muscles and bones... but under microscope)

- in (Greek): "Histo" means : "tissue" , "logos" means "study of "

- the smallest functional unit in our body : the cell

- cell → tissue → organ → system → organism

* Group of cells (have the same structure and function) = tissue

  - (4 kinds of tissues : connective, epithelial, nervous, muscular)

* Group of tissues (connected together) = organ

  - (ex : stomach ; with lining inside stomach =epithelial , muscles that contracting food = muscular, muscles are controlled by nerves = nervous , all of these tissues are connected by connective tissue)

* Group of organs = system

  - (gastrointestinal الهضمي : stomach , esophagus مريء , intestine أمعاء … and respiratory system)

* Group of systems = organism (mouse …)

- Bichat was the first anatomist that defined tissue without microscope.

- you have to know these **units** in histo :

* 1mille meter = 1000 micro meter (µm)
* 1µm = 1000 nano meter (nm)
* 1mm=1 000000 nm

Micro techniques

- Micro techniques (or histological Micro techniques):"How to prepare a tissue in order to view it under the microscope".

- Microtechniques have different methods and the same basics(to harden the tissue in order to section it) .

- We have to ways two hardening the tissue :
  1) paraffin wax (take 12 hours)
  2) freezing (20 min)

1) paraffin wax: to replace water with molten wax then to harden it producing a block of tissue **.**

a) **fixation(تحنيط):** by fixative agent " formalin" (paraformaldehyde) , in order to preserve the tissue's structure and shape from bacteria and enzymes . time depends of tissue's size .

b) **dehydration** in order to remove the water (by putting the tissue inside ascending concentration of alcohol 70% , 80% …. 100%)

-we don't put it directly in 100% alcohol because the tissue will shrink and the structure will be different **.**

c) **clearing** : in this step we remove alcohol and replace it by xylene (an organic solvent) in order to replace the xylene by the wax (because alcohol doesn't dissolve in the wax) .

d) embedding : to put the tissue within a block by putting it inside a small box containing a molten wax in order to get a hard block .

e) sectioning : we use microtome (wheel with a handle, knife, block holder) which cuts the tissue into micrometer thickness here in order to produce ribbon of sections .

- after putting the block in a block holder , we start to turn the wheel (each turn of the wheel the tissue moves up and down toward the knife) , with the result that we will get sections. (the wax and the tissue is inside)
- we can control the thickness of the sections

f) mounting : to put the section over a glass slide after putting it in water path in order to make it flat **.**

g) staining : in order to view under microscope **.**

h) cover the slide with a cover slip **.**

❖ **Advantages:** high quality sections , complete sections , series of sections .

❖ **Disadvantages:** it needs 12 hours (long time) , difficult to study fats and lipids

2)Freezing

And it has the same concepts

a) Hardening by freezing instead of using wax .
b) Then we put the sample inside liquid nitrogen
c) Now , we have a hard tissue , and cut it by special type of microtome called cryostat (has the same parts of microtome)which is placed inside freezer , we control the temperature less than 0 .
d) Then we have a section , stain it and so on .

❖ **Advantages :** this way is very fast (20 min)

❖ **Disadvantages :** producing poor quality of sections

- **Differences between the two ways :**

- in Paraffin wax we use heat and xylene (organic solvent can dissolve lipids)
- in order to preserve fat and lipids you must use freezing technique (heat and xylene destroy it)
- Freezing technique is used in surgical procedure (to have quick result).

Staining

❖ types of staining :

1) Ordinary stains : for example (eosin , hematoxylin)
2) Direct and indirect Immunohistochemistry
3) Hybridization

- **Immunohistochemistry :**

This method depends on the interaction between the antibody and antigen.

a) **Direct IHC :** to study a specific protein called antigen you must determine the location of this protein by an antibody which interacts with specific antigen .

ex :

-The protein(antigen) here is actin and you use a specific antibody in order to interact with this antigen.

- how to produce this antibody ?

inject a specific antigen (here : actin)in an animal, this will induce animal's immune system to produce antibodies because antigen will be considered as a foreign body , then some of the blood is taken and gets purified in order to get antibodies.

- Now, you have specific antibody for the antigen

- (you have the cell and you will now localize the location of protein) → put labeled antibody (by fluorescent material) on the tissue → put the section under fluorescent microscope which detect the color's signal .

e) Indirect IMH

- The same concepts (antigen > antibody>cell ….) but the antibody is not labeled which is called (primary antibody), and we will use another antibody called secondary antibody (labeled by a fluorescent material) to bind with the primary antibody .
- We do this because more than one secondary antibody can interact with primary antibody (but primary has only one binding site at the antigen) and this will make viewing the protein easier because more fluorescence will emit from interaction .

* IMH is used to localize protein .

- **Hybridization**

  - The same process but instead of looking for proteins we look for a certain sequence inside the gene (DNA).we produce a complementary for the virus genes .

* For example, we took a sample of saliva and we want to see if the saliva is infected with a certain virus, so we search for the sequence of the virus DNA or RNA by using a small oligonucleotides which are complementary to the target DNA or RNA sequence, we make the complementary sequence in the lab by making a complementary sequence for the virus, the sequence should be probe with a signal (fluorescence material). Then, when we apply heat for the sample it denaturates, then we remove any excess. After using microscope,if there is a signal then the saliva is infected.

Microscope

-There are 2 main types of microscopes: light microscopes and electron microscopes.

A) Light microscope (LM) :

1. bright-field microscope:

* max magnification is 1500X

- (x means : how times this lense magnificates the section)

-(overall magnification = magnification of ocular * magnification of objective)

*tissues are dead because of used stains

*this microscope has a tube(most important part) with oculars and objective lenses , stage(to put the section) and arm(to carry it)

2.phase contrast microscope:

* tissues are alive here:Cells should be grown in the lab using growth materials in an incubator with tissue culture

* the image has different contrasts because light changes its speed and direction when passing through different materials.

* By using this microscope, you will be able to determine the overall shape of the cell without using stains.

3.fluorescence microscope:

ultraviolet light is used instead of visible light, tissues are marked with a fluorescent material which emits fluorescence.

4.confocal microscope (recent type):

* it is connected to computer

* laser beam is used in this microscope, thickness of sections is 10µm usually but in this microscope's software can make multiple layers from this 10µm section

* this microscope produce 3D images and more contrast than fluorescence microscope.

B)electron microscope:

* uses electron beams , electrons can't pass through stained areas so stained areas appear black and veca versa .

* no wax is used because sections are ultra thin so electrons will not be able to penetrate the section

* copper mesh is used instead of glass slide, sections are prepaed by ultramicrotome, lenses are electromagnet, knives used are made from diamond and glass not metal , sections are stained with heavy metals.

- Transmission electron microscope (TEM) is used to study ultra structure of the cell and used main in histology
- while Scanning electron microscope is used to study the surface of the cell with a 3D image, the image is 3D because electrons are deflected and caught by a screen, and used mainly in biology .

	LM	EM
Image	Colored (stained)	Black and white
Image is produced by ….	Visible light rays	Electron beams
Magnification*	1500X	2×10^{6}X
Resolution*	0.25 µm	0.1 nm
time	20 mins for frozen	1 day

	sections	
Thickness of sections	1-30 µm Usually 10µm	0.02-0.1µm
Place of specimen	Glass slide	Copper mesh

***magnification is the ratio between image size and real size**

***resolution is the minimum distance 2 points can be separated and still be distinguished as separate points**

Histology is looking at two dimensional image of a three dimensional structure

GOOD LUCK