Cell Biology

lecture 2

L2: Microtechniques

4/Nov/2024

Cell-Biology , L2: Microtechniques
التقنيات المجهرية

Microtechniques for light microscopy

↓

To preserve the structure & Chemical Composition of the specimen

- **Parffin** I
- **freezing** II

(I) Paraffin technique :

- its a technique used to **Prepare the tissues** for **light microscopy**

-Steps : [Must Knew in order]

1. **Fixation** : in appropriate solution (**formol Saline**)
2. **Dehydration & Clearing** : in **alcohol** then **xylol**
3. **impregnation & Embedding** : in **Paraffin wax** (soft & hard)
4. **Sectioning** : by **microtome**
5. **Mounting** : on **glass slides**
6. **Staining** of the sections

the protocol : Receipt & identification ⟹ Labeling specimen ⟹ Fixation ⇓

Dehydration ⟸ Clearing ⟸ impregnation ⟸ Embedding ⟸ sectioning ⇓

staining ⟹ Mounting

① fixation

- to maintain the structure of the tissue as in the life state

- after removing /extracting the sample from the body its immediately put in a fixative solution
محلول مثبت

.Fixation is done as soon as possible to Prevent autolysis & Preserve morphology

- The solution used for:

*LM (light microscope):
is formal saline with 10% concentration in solution

Ex: 1000 ml
100 ml formal saline
900 ml water

*EM (electronic microscope):
is used a mixture of
Glutaraldehyde
& osmium tetroxide

*Advantage of this fixation step:

- Hardens the tissue by Coagulating (تخثر) its Protein which facilitates the process of cutting & staining & Exmination

- Prevents putrefaction & stops autolytic changes by killing any bacteria
(تعفن، تحلل)

- Preserves the molecular & morphological structure of the tissue

(2)

Dehydration & Clearing

Dehydration

- This is done by:
  treating the specimen with ascending concentration of alcohol (50% → 70% → 100%)
  * This is to gradually remove water from the specimen preserving its shape
  [H_2O represents 70% of the body]

Clearing

- This is done by:
  treating the specimen with xylol or benzol to remove the alcohol from the dehydration
  * This process makes the tissue of the specimen become translucent
  [allowing light to penetrate the specimen]

(3)

Impregnation & Embedding

Impregnation

- Tissues are placed in: molten Soft Paraffin wax
- The wax infiltrates the tissue & occupies all the spaces that were occupided with water in the beginning

Embedding

- Tissues are placed in: molten Hard Paraffin wax
- The tissue is placed in: the center of the paraffin which hardens as it cools so we get a Paraffin block
- This prevents tissue damage
- the specimen orientation is important

④ Sectioning by Microtome

↳ a Mechanical device
used to cut extremely thin slices
of a fixed tissue block
[known as: sections]

- it holds the hard paraffin block with the tissue in its center against a sharp metal knife that is used to cut the block into thin sections [3–10 microns] as it moves up & down. This gives a serial sectioning

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⑤ mounting :

- Tissue sections are placed on :
  Glass slides smeared with egg albumin
- Then its warmed on a hot plate to dry
- and now the sections are ready to be stained

(II) Freezing technique

- its to preserve the chemical composition of the tissue

★ Fresh frozen tissues are cut using: Cryostat
[freezing microtome]

- the sections are placed in a cold fluid

Isopentane or Liquid Nitrogen

[-50°C]

for fixation & then rapidly stained

Advantages:

- Rapid technique for diagnoses in operation rooms (tumors)

- There is NO fixation nor dehydration & NO chemicals are used and that is useful for histochemical studies [enzyme staining]

Disadvantages:

- Non-Serial & fragmented sections

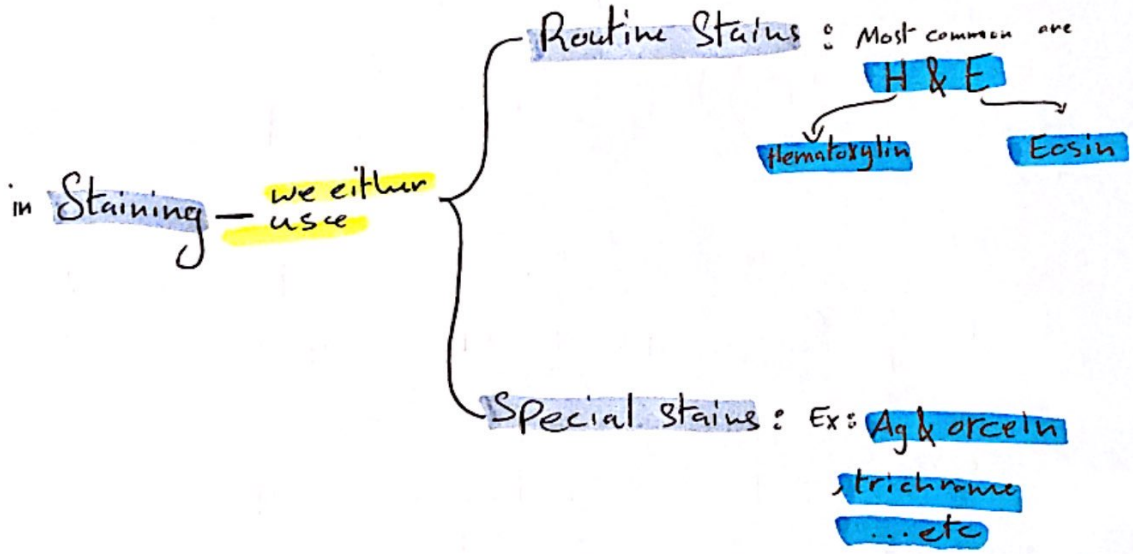
- Cannot be preserved for a long time

★ Staining ★ : used to visualize & distinguish different parts of cells and tissues

- when studying tissues is by using Light Microscope they must be stained first bc most tissues are colorless

- The Dyes or Stains used are either:

acidic or basic



Common [Routine] histological stains H & E

Hematoxylin (H)

* Blue & Basic dye
* (+ve charged)
* Stains acidic (anionic -ve) components of the cell like:
  [Nucleus, Ribosomes (r-RNA)] ← bc of the Phosphate group
  with a blue color
* Basophilic structure = blue

Eosin (E)

* Red & Acidic dye
* (-ve charged)
* stains basic (cationic +ve) components of the cell like: [Cytoplasm, mitochondria, muscles]
  they have +ve charged Proteins
* Acidophilic structure = red

* Take a look at Pictures on slide 14

- The clinical value of Special Stains :

- They answer specific questions like:
  what type of cells and tissues

- Are used in the diagnosis of medical diseases like:

Trichrome Stain , in case of Liver Cirrhosis
(we use this stain in case of this disease diagnosis)

* Look at pics on slide 19

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① Vital Stain : - Tests the viability of the cell (حيوية الخلية)

- It stains Living cells inside the living animal

* done by : injecting the dye into the living animal prior to examine the tissue

Ex: Staining Phagocytic cells [Macrophages (خلايا البلعمة)]
with : Trypan blue & Indian ink
(Macrophages → found in connective tissue)

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② Leishman Stain : - A mixture of acidic & basic stains

- used to ~~stain~~ stain : [Nuclei & Cytoplasm]
  using : [Methylene blue] & [Eosin]

- also used to stain blood films
  and thats to demonstrate white blood cells
  Ex: malaria Parasite

* Look at pics on slide 20 & 21

③ Metachromatic Stain:

- Its a stain that gives the tissue a color that is different from the color of the stain itself → in connective tissue

Ex: when staining [Mast cells] with [Toluidine blue] it gives Purple color (the stain itself is blue but gives Purple)

and this phenomenon is called: [Metachromasia]

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④ Trichrome Stains: (Connective tissue)

- They are 3 stains that are used in combination to stain different tissue components

Ex: [Collagen Fibers] are stained Blue

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⑤ Orcein Stain:

- Stains: [Elastic fibers] with brown
  → like wall of aorta

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⑥ Silver (Ag) stain:

- Stains: [Nerve cell] with brown
  & [Reticular fibers] in black

* See pics

✱ Histochemical Stains : [Relate structure to function]

- Its a technique used to selectively identify & demonstrate the distribution of chemical compounds or enzymes both within & between the cells

Ex: [mucine or alkaline Phosphatase enzyme] ↴

removes Phosphate group from Protein

Concept: Enzyme of interest in a cell or a tissue converts its substrate into: Colored or florescent product that can be visualized at the site of the activity

✱ we use the freezing technique with those [enzymes]

* Immuno-histochemical (IHC) stains :

- Its a laboratory method that:
  selectively identify antigens

and that is using specific ~~antibodies~~ antibodies to check for these antigens in a sample of tissue

- The antibodys are usually linked to an enzyme or a florescence dye (markers) and called [Labeled antibodies]

- After the antibodies are linked to the antigen in the tissue sample, the enzyme or dye is activated and the localization of the antigen can be seen under the microscope

- This method is used to visualize both Normal & disease status of tissue

- also used in diagnosis of cancers (markers) and it can tell the difference between different types of cancer [Tumor Specific]

* Indirect vs direct (IHC) :

Direct → ideal for detecting highly expressed antigens

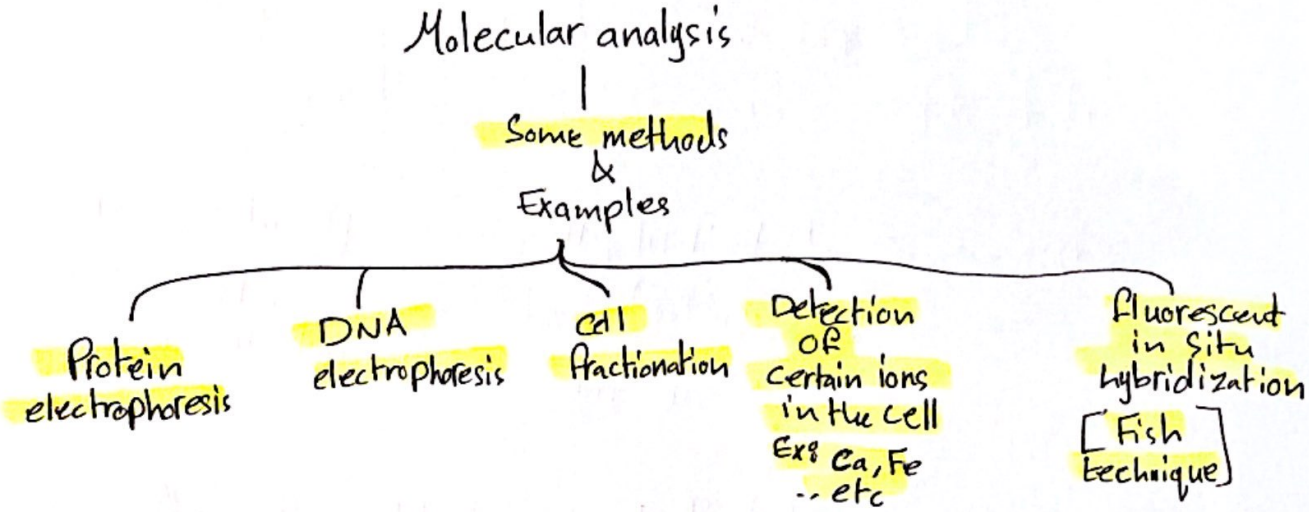
more suitable for poorly expressed antigens but benifited signal amplification by the secondary antigen

Indirect (simple)

Look at Pics

* Molecular analysis

- It means biochemical analysis of certain components of the cell
- it is usually quantitative in nature



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① Protein electrophoresis

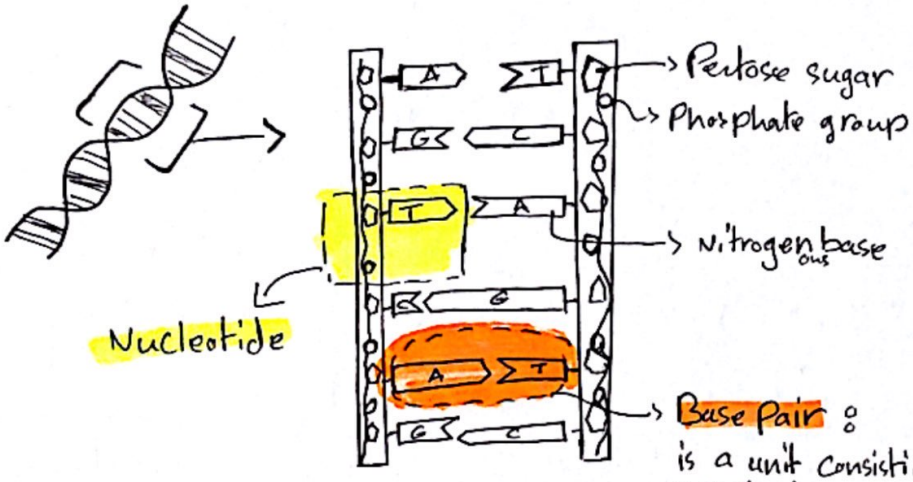
* Proteins carry a positive or negative electrical charge
  - So they move in fluid when placed in an electrical field
  - They will be seperated according to their Charge & Molecular weight

  Ex: blood Cancers [multiple myloma]
  - it increases the : gamma globulin protein (M Protein) and thats how its diagnosed

② DNA electrophoresis :

- its a technique used to : Identify & Quantify DNA fragments

* DNA fragments are (-ve charged)



- the human genome has approximately 3 billion of these base pairs that reside in the 23 chromosome pairs within the nucleus of all the cells

- They form the building base of the DNA Double helix

- sequence of bases on DNA determine the genetic code for a trait

* So in this case, seperation is based on : the length of the base pair

- samples are loaded into wells of an [Agarose or Acrylamide gel] then subjected to an electric feild

- this electric field causes the negatively charged nucleic acids to move towards the positive electrode and small fragments will move faster therefore for more distance away from the negative electrode

-Look at pics on slides :>

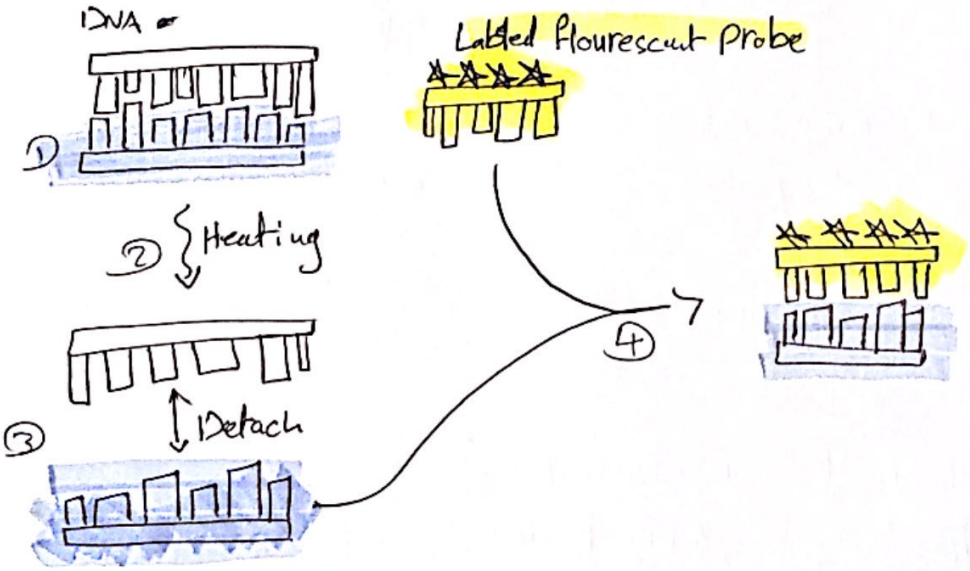
*So this is used in :

- DNA fingerPrinting
- Gene isolation
- disputed paternity

③ Flourescent In Situ hybridization [Fish Technique]:

- In Situ → Localized
- hybridization → Binding

- It's a molecular technique used to visualize & map the genetic material useful to diagnose genetic diseases & studying Chromosomes
- It's used to localize the site of the genes on Chromosomes using: a [flourescent Probe]
  - It's a fragment of DNA or RNA of variable length that can be radioactively labeled (Probe)



- it can be used in DNA or RNA samples to:
  Detect the presence or absence of a nucleotide sequence that is complementary to the probe
  - useful to detect chromosomal abnormalities

Methods for studying tissues

In vivo studies
[within the living body]

study of tissues after doing any experiment inside the living body (animal model based testing)

In vitro studies
[outside the body]

study of tissues outside their normal biological context (cell based testing)

★ Cell and tissue culture :

- In vitro :
- Cultivation of tissues & cells at a defined temperature (37°C) using an incubator and supplemented with a medium containing cell nutrients & growth factors like: [animal serum]

this is known as [tissue culture]

- Different types of cells can grow in cultures as :
[white blood cells, fibroblasts, skeletal & cardiac muscle, epithelial tissue (liver, breast, skin, kidney), and many different types of tumor cells]

Medical uses of tissue culture

- Studying Chromosomal Patterns of individuals
  - Karyotyping
  - gene therapy
- researches of Cancer
- Cultivation of bacteria, viruses, in order to Prepare different vaccination
- Study the effects of new drugs

*Cell culture:

- Cells can be isolated outside of the body for in vitro cultures
- they can be released from tissues by enzymatic digestion using Enzymes such as [Collagenase] & [Trypsin] which break down the extracellular matrix.

- Primary cultures:

Refers to the cells that are cultured directly from a tissue (Parent cells)

- Secondary Cultures:

once Parent cells reach confluence they have to be sub-cultured (Passaged) by transferring them to a new vessel with fresh growth medium to provide more room for continued growth

- Confluence:

Stage in which cells (1ry or 2ry) become [adherent] to & covering most of the culture surface forming monolayer

(adherent: means attached to)

- Cell line:

- a population of cells (clones) developed from a single cell therefore they have a uniform genetic make up (Phenotype & function)
- Cell lines have a limited life span as they are passaged

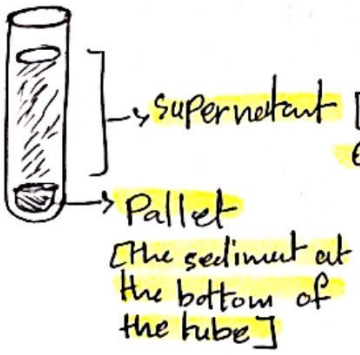
* immortalized cell line:

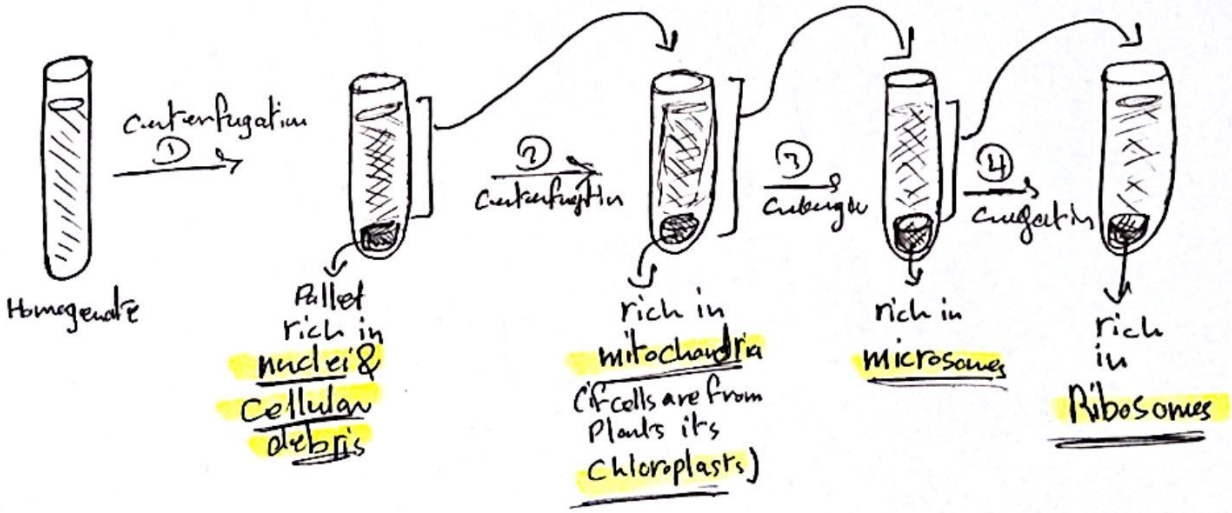
- has acquired the ability to Proliferate indefinitely
- is obtained from subcultures of the primary culture
- Normal immortalized cell line: Stem cells
- Abnormal immortalized cell lines: Cancer cells

✱ Cell fractionation :

- means isolation of cell components (nucleus & organells) while preserving its individual function to study the features of each

- This is done by the use of [centrifugation] at different speeds and periods of time
  - The factor that determines wheather a specific cell component ends up in the supernatant or the pallet is size & weight of component
  - Nuclei is the first to be seperated followed by different cell organells





✱ memorize in order ✱