MEDWAY
ACADEMY
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QUESTION BANK

SEMESTER 1

TISSUE STRUCTURE FUNCTION (TSF)

Lecture 1 - Micro-techniques

Part 1: MCQs (Choose the correct answer):

1. A first-year student is asked about the level of organization of the body in which many cells are held together by a glue-like intercellular substance to act together and perform a common function. Which of the following is the correct level?

A. Chemical level of organization
B. Cell
C. Tissue
D. Organ
E. System

2. A 43-year-old man came to the clinic complaining of a swelling in the skin of his cheek. A biopsy was taken and was immediately immersed in formaldehyde before being sent to the histology laboratory. What is the aim of this step?

A. Removal of the water from the tissue
B. Cross-linking of the proteins and killing of the bacteria
C. Making the tissue transparent to the light
D. Hardening of the tissue inside a wax block
E. Colouring of the nucleus and the cytoplasm

3. During the processing of a fixed piece of liver, the technician passes the specimen through ascending grades of alcohol up to 100% alcohol. What is the aim of this step?

A. Removal of the water from the tissue
B. Removal of the alcohol from the tissue
C. Prevention of the autolysis of the tissue
D. Detection of the enzymes of the tissue
E. Increasing the contrast of the section

4. After being passed through absolute alcohol, a specimen is immersed in xylene before being put in soft paraffin. What is the aim of using xylene?

A. Fixation of the tissue proteins
B. Staining of the basophilic structures
C. Mounting of the section on the glass slide
D. Removal of the alcohol so that the wax can penetrate the tissue
E. Freezing of the tissue before cutting

5. A specimen has just been impregnated with soft paraffin at 58–60 °C. Which of the following is the next step in the paraffin technique?

A. Fixation in formaldehyde
B. Dehydration in alcohol
C. Clearing in xylene
D. Embedding of the tissue in a paraffin wax block
E. Staining with haematoxylin and eosin

6. A technician is cutting sections 5–10 µm thick from a wax block containing a piece of kidney. Which of the following instruments is he using?

A. Cryostat
B. Ultramicrotome
C. Condenser
D. Revolving nose piece
E. Microtome

7. A researcher wants to demonstrate the activity of the dehydrogenase enzymes inside the mitochondria of a muscle biopsy. Why is the paraffin technique unsuitable for this purpose?

A. Because the paraffin sections are too thin to be examined
B. Because the paraffin sections cannot be mounted on a glass slide
C. Because the heat and the solvents used destroy the enzymes and dissolve the fat
D. Because the paraffin technique takes only a few minutes
E. Because the paraffin sections cannot be stained with H & E

8. A fresh specimen is frozen in liquid nitrogen and thin sections are cut and stained within a few minutes. Which of the following instruments is used to cut these sections?

A. Microtome
B. Cryostat
C. Condenser lens
D. Fine needle and syringe
E. Scanning electron microscope

9. A section of the pancreas stained with H & E shows a deeply blue-stained basal part of the cytoplasm of the acinar cells. Which of the following cell components is responsible for this appearance?

A. Secretory granules
B. Mitochondria
C. Rough endoplasmic reticulum
D. Smooth endoplasmic reticulum
E. Golgi apparatus

10. Cells that are packed with mitochondria appear deeply pink in a section stained with H & E. Which of the following explains this appearance?

A. Haematoxylin is a basic dye that stains the basophilic structures
B. Mitochondria are rich in phosphate groups
C. Eosin is an acidic dye that stains the acidophilic structures
D. Eosin is a basic dye that stains the basophilic structures
E. Mitochondria are stained magenta by PAS

11. A liver biopsy taken from a child suspected of having a glycogen storage disease is stained with PAS. What is the colour of the stored glycogen?

A. Black
B. Magenta
C. Orange
D. Blue
E. Green

12. A pathologist wants to demonstrate the fat droplets inside the hepatocytes of a patient diagnosed with fatty liver. How should the specimen be prepared and stained?

A. Paraffin sections stained with H & E
B. Paraffin sections stained with PAS
C. Frozen sections stained with Sudan III
D. Paraffin sections stained with Azan
E. Frozen sections stained with silver

13. A section of the skin is stained with Azan stain. What is the colour of the collagen fibres of the dermis?

A. Blue
B. Red
C. Black
D. Magenta
E. Orange

14. A researcher wants to demonstrate the reticular fibres in a section of a lymph node. Which stain should he use, and what colour will these fibres appear?

A. PAS; they appear magenta
B. Sudan III; they appear orange
C. Azan; they appear red
D. Staining with silver; they appear black
E. Haematoxylin; they appear blue

15. A section of the kidney is treated in the laboratory to demonstrate the activity of the phosphatase enzymes. This type of staining is called:

A. Immuno-histochemical staining
B. Routine haematoxylin and eosin staining
C. Histochemical staining
D. Silver staining
E. Azan staining

16. Labelled antibodies are applied to a tissue section in order to identify and localize a specific protein. This technique depends on which of the following?

A. A reaction between an acidic dye and a basic structure
B. An antigen–antibody reaction
C. A reaction between an enzyme and its substrate
D. Reflection of the electrons from the coated surface
E. Cross-linking of the tissue proteins by the fixative

17. A 48-year-old woman is diagnosed with breast carcinoma. Immunohistochemistry of the biopsy reveals abundant oestrogen receptors in the tumour cells. What is the main value of this result?

A. It proves that the tumour is benign
B. It measures the size of the tumour
C. It demonstrates the fat content of the tumour cells
D. It determines the line of treatment of the patient (anti-oestrogen)
E. It gives a three-dimensional image of the surface of the tumour

18. A 40-year-old woman attends the clinic for screening. Epithelial cells are removed from her uterine cervix by a plastic brush and are spread on a glass slide. This method of sampling is called:

A. Aspirated cytology
B. Needle biopsy
C. Exfoliated cytology
D. Freezing technique
E. Tissue culture

19. Which of the following is a use of cytology?

A. Preparation of ultrathin sections for the electron microscope
B. Cultivation of viruses for the production of vaccines
C. Demonstration of the three-dimensional surface of the cilia
D. Detection of microorganisms in a cervical smear
E. Prevention of the putrefaction of the tissue

20. Cells obtained from the umbilical cord of a newborn are kept alive outside the body in order to study the effect of a new drug on them. Which of the following is another use of this procedure?

A. Rapid diagnosis during a surgical operation
B. Staining of the collagen fibres in blue
C. Detection of the fat inside the liver cells
D. Increasing the contrast of the ultrathin sections
E. Cultivation of viruses and production of vaccines

21. Which of the following microscopes is considered an enhancement of the fluorescence microscope because it excludes the light that is out of focus?

A. Phase contrast microscope
B. Confocal microscope
C. Transmission electron microscope
D. Scanning electron microscope
E. Ordinary light microscope

22. What is the limit of resolution of the ordinary light microscope?

A. 0.1 nm
B. 0.2 µm
C. 5–8 µm
D. 60–80 nm
E. 1 mm

23. In the scanning electron microscope, the image of the specimen is produced by measuring which of the following?

A. The differences in the transparency of the specimen to the electrons
B. The amount of light absorbed by the stained section
C. The fluorescence emitted by the specimen
D. The thickness of the paraffin section
E. The differences in the reflection of the electrons from the surface

24. Ultrathin sections (50–100 nm) prepared for the transmission electron microscope are stained with heavy metals such as osmium tetroxide. What is the aim of this step?

A. To prevent the autolysis of the tissue
B. To dissolve the fat of the tissue
C. To increase the contrast of the section
D. To keep the cells alive during the examination
E. To make the sections thicker

25. Which of the following is correct as regards the electron microscope when it is compared with the ordinary light microscope?

A. It uses a beam of light that travels through the air
B. Its lenses are made of glass
C. The tissue is embedded in paraffin wax
D. It uses a beam of electrons that travels through a vacuum
E. Its resolving power is 0.2 µm

Part 2: Short-Answer & Essay Questions:

1. A 43-year-old man is complaining of a mass in the skin of his face. An excisional biopsy is taken and sent to the histology laboratory to be prepared as a paraffin section.

a. Enumerate, in order, the steps of preparation of a paraffin section.

b. Mention the aim of the fixation and the aim of the clearing.

c. The doctor then asks for the demonstration of the enzymes of this tissue. Mention the suitable technique, and explain why the paraffin technique cannot be used.

2. Compare between the paraffin technique and the freezing technique.

	Paraffin technique	Freezing technique
Time of preparation		
Quality of the section		
Uses		

3. Compare between the transmission electron microscope (TEM) and the scanning electron microscope (SEM).

	Transmission electron microscope	Scanning electron microscope
Preparation of the specimen		
What the microscope measures		
The image obtained		

4. Haematoxylin and eosin (H & E) is the routine stain used in histology. Complete the following:

A. Haematoxylin is a …… stain (acidic or basic), and it stains the …… structures of the cell. Mention two examples.

B. Eosin is a …… stain (acidic or basic), and it stains the …… structures of the cell. Mention two examples.

5. A 6-year-old boy is investigated for an enlarged liver, and a liver biopsy is taken. The pathologist wants to demonstrate both the glycogen and the fat inside the hepatocytes.

A. Which stain is specific for the glycogen, and what colour does it give?

B. Which stain is used for the fat, and how should the tissue be prepared before staining?

C. Mention one other special stain and its use.

6. A 45-year-old woman is complaining of a swelling in her thyroid gland. Cells are withdrawn from the swelling by a fine needle and a syringe and are spread on a glass slide.

A. What is the name of this method of cytological sampling? Mention the other method of cytological sampling with one example.

B. Mention two uses of cytology.

C. Define immuno-histochemistry and mention one of its uses.

Part 1: MCQ Answer Key (with slide references)

1. C – Tissue Slides 5 & 6 (many cells held together by intercellular substance to perform a common function)
2. B – Cross-linking of the proteins and killing of the bacteria Slide 10 (fixation by formaldehyde prevents autolysis and putrefaction)
3. A – Removal of the water from the tissue Slide 10 (dehydration by ascending grades of alcohol up to 100%)
4. D – Removal of the alcohol so that the wax can penetrate the tissue Slide 10 (this step is called clearing)
5. D – Embedding of the tissue in a paraffin wax block Slide 10 (sampling → fixation → dehydration → clearing → impregnation → embedding)
6. E – Microtome Slide 11 (the wax block is cut into sections 5–10 µm thick)
7. C – The heat and the solvents used destroy the enzymes and dissolve the fat Slide 14 (the freezing technique is used instead)
8. B – Cryostat Slide 14 (sections are cut and stained within a few minutes)
9. C – Rough endoplasmic reticulum Slide 12 (its ribosomal RNA is basophilic and is stained blue by haematoxylin)
10. C – Eosin is an acidic dye that stains the acidophilic structures Slide 12 (membrane components of mitochondria and sER, cytoplasmic filaments and secretory granules)
11. B – Magenta Slide 13 (carbohydrates such as glycogen are stained by PAS)
12. C – Frozen sections stained with Sudan III Slides 13 & 14 (the paraffin technique dissolves the fat; Sudan III gives an orange colour)
13. A – Blue Slide 13 (Azan stains the nuclei red and the collagen blue)
14. D – Staining with silver; they appear black Slide 13 (reticular fibres are argyrophilic)
15. C – Histochemical staining Slide 13 (histochemical staining detects the enzymes in the tissues)
16. B – An antigen–antibody reaction Slides 13 & 15 (immuno-histochemistry identifies and localizes specific proteins)
17. D – It determines the line of treatment of the patient (anti-oestrogen) Slide 16 (individualized therapy; the same applies to the progesterone and HER2 receptors)
18. C – Exfoliated cytology Slide 17 (cells shed or scraped from body surfaces)
19. D – Detection of microorganisms in a cervical smear Slide 18 (also diagnosis and screening for malignancy)
20. E – Cultivation of viruses and production of vaccines Slide 19 (also the study of cell growth, differentiation and the effect of new drugs)
21. B – Confocal microscope Slide 20 (an enhancement of the fluorescence microscope)
22. B – 0.2 µm Slides 20 & 25 (the resolving power of the electron microscope is 0.1 nm)
23. E – The differences in the reflection of the electrons from the surface Slide 24 (the specimen is first coated with a thin film of metal (platinum))
24. C – To increase the contrast of the section Slide 24 (TEM measures the transparency of the specimen to electrons)
25. D – It uses a beam of electrons that travels through a vacuum Slide 25 (its lenses are electromagnetic, the embedding medium is epoxy resin and the final image is projected on a fluorescent screen)

Part 2: Short-Answer & Essay Answer Key (with slide references)

1. Paraffin section of a skin mass — Slides 10 & 11

- a) Sampling of a small piece of the tissue → fixation in formaldehyde → dehydration in ascending grades of alcohol up to 100% → clearing in xylene → impregnation with soft paraffin (58–60 °C) → embedding in a paraffin wax block → cutting of sections 5–10 µm thick by the microtome → mounting on a glass slide → staining of the section.
- b) Fixation: the fixative (formaldehyde) cross-links the proteins and so prevents autolysis, and kills the bacteria and so prevents putrefaction. Clearing: removal of the alcohol from the tissue so that the paraffin wax is able to penetrate it.
- c) The freezing technique. The paraffin technique is not suitable for the demonstration of the enzymes or the fat, because they are destroyed and dissolved during the processing.

2. Paraffin technique versus freezing technique — Slides 8, 10, 11 & 14

- Paraffin: takes 1–2 days; gives thin, well-stained sections; it is the most common (routine) technique used for diagnosis.
- Freezing: takes only a few minutes; the section is less well stained; used for rapid diagnosis during a surgical operation and for histochemistry (detection of enzymes and fat).

3. TEM versus SEM — Slide 24

- TEM: ultrathin sections (50–100 nm) stained with heavy metals (osmium tetroxide) to increase the contrast; it measures the relative differences in the transparency of the specimen to the electrons; it gives an image of the interior of the cell.
- SEM: the surface of the specimen is coated with a thin film of metal (platinum); it measures the relative differences in the reflection of the electrons; it gives a three-dimensional image of the surface.

4. Haematoxylin and eosin — Slide 12

- A. Haematoxylin is a basic (positively charged) stain; it stains the basophilic (negatively charged, phosphate-containing) structures, e.g. the DNA and RNA of the nucleus, the ribosomes, the rough endoplasmic reticulum and the cartilage matrix.
- B. Eosin is an acidic (negatively charged) stain; it stains the acidophilic structures, e.g. the membrane components of the mitochondria and the sER, the cytoplasmic filaments and the secretory granules.

5. Special stains in a liver biopsy — Slides 13 & 14

- A. PAS is specific for the carbohydrates (glycogen); it gives a magenta colour.
- B. Sudan III (orange), Sudan black (black) or osmic acid; the tissue must be prepared as frozen sections, because the paraffin technique dissolves the fat.
- C. Any one of: Azan (nuclei red, collagen blue); silver (reticular fibres black); histochemical staining (detection of enzymes); immuno-histochemical staining (Ag–Ab reaction).

6. Cytology of a thyroid swelling — Slides 15–18

- A. Aspirated cytology (tumours and body fluids). The other method is exfoliated cytology, which uses cells shed or scraped from body surfaces, e.g. bronchial cells coughed up in the sputum, or cervical cells removed by a plastic brush.
- B. Diagnosis of malignancy (e.g. bronchial carcinoma in the sputum); screening for malignancy (e.g. CIN in the cervical smear); detection of microorganisms.
- C. Immuno-histochemistry: a staining method based on the antigen–antibody reaction, in which labelled antibodies are used to identify and localize specific proteins. Uses: demonstration of cytoplasmic components, diagnosis of tumours (lymphomas) and the choice of therapy in breast cancer.