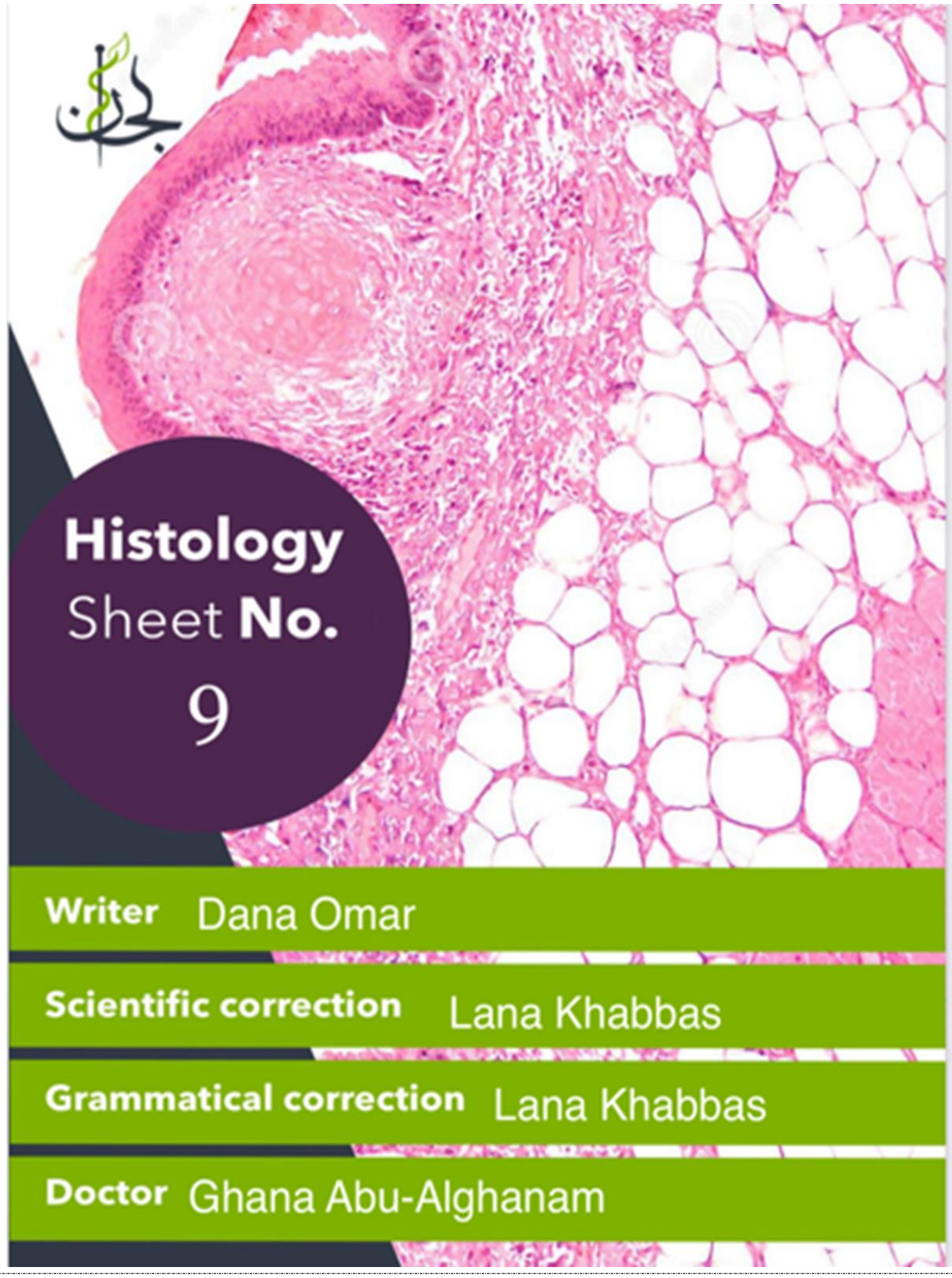


Histology
Sheet No. 9

Writer Dana Omar

Scientific correction Lana Khabbas

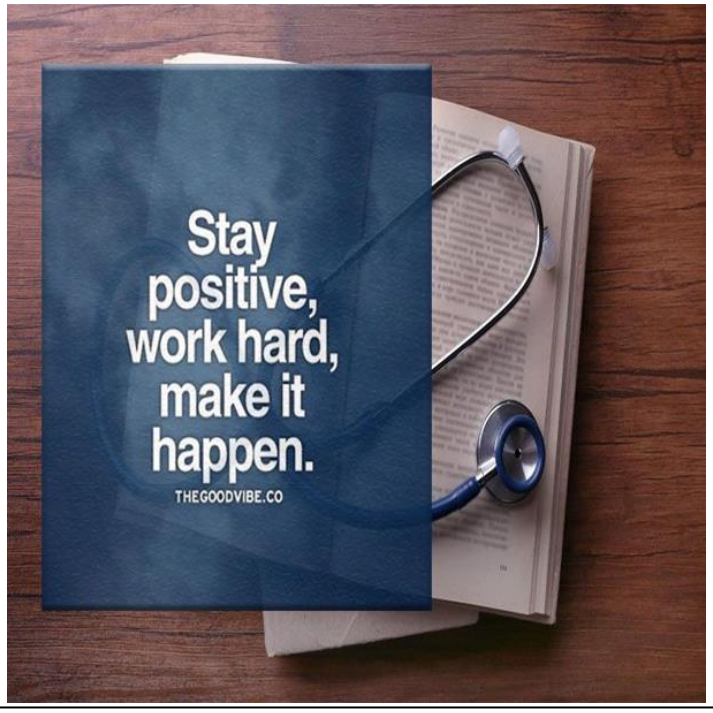
Grammatical correction Lana Khabbas

Doctor Ghana Abu-Alghanam

Dear colleagues, you have to know some notes:

1.The Black colour represents Dr.Ghana notes in the lecture.
2.The Green colour represents information in Junqueira's histology book+ Dr.Hanan notes in her slides.
3.The photos taken from the book, you have to look at the box under it, has many informations.

#some notes are taken from Google to deep understanding.



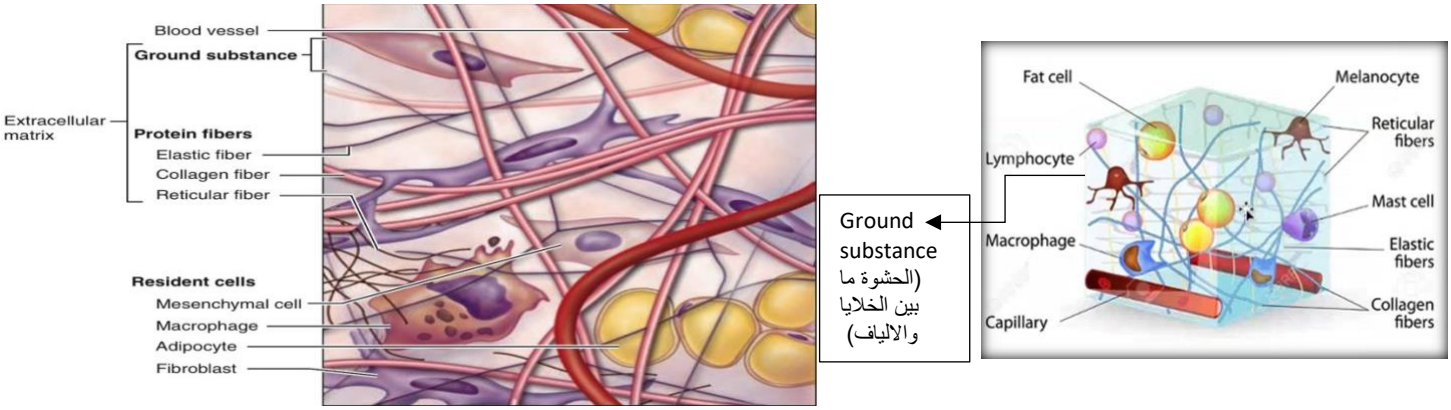
Believe in yourself, believe in the person that you will become.

We talked about the epithelium tissue in the last lectures, and now we will talk

Connective Tissue.

Connective tissue could be classified to two different types; general and special (consists of reticular connective tissue, adipose tissue, cartilage, bone, and blood). It is almost present in everywhere in your body! It's mainly composed of 3: cells, (protein fibres, and ground substance) the last two are the ECM.

The rest of the functions of the epithelium tissue (examples of epithelium tissue's functions: conduction and contractility of muscles and nerves) is usually provided by the connective tissue

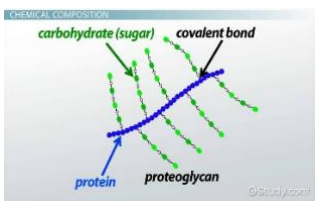


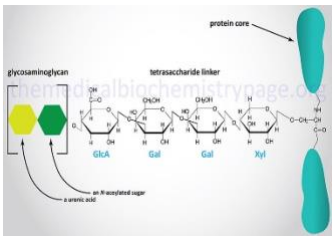
Sometimes the difference between the special types is in the nature of the ground substance (ground substance is a component of the matrix with the protein fibres), for example, the bones (connective tissue) its ground substance is very hard, whereas in the cartilage even it is also a special type ,it's ground substance is most likely rubbery or elastic.

- Connective tissue provides a matrix that supports and physically connects other tissues and cells together to form organs of the body.
- The interstitial fluid of connective tissue gives metabolic support to cells as the medium for diffusion of nutrients and waste products.

Ground substance is a complex of anionic, hydrophilic (because its fluid) proteoglycans, glycosaminoglycans (GAGs) which are polysaccharides consisting of repeating disaccharide units. The repeating two-sugar unit consists of a uronic sugar and an amino sugar, and multiadhesive glycoproteins (laminin, fibronectin, and others).Made by fibroblasts.

- Within the ground substance, water allows the exchange of nutrients and metabolic wastes between cells and the blood supply.

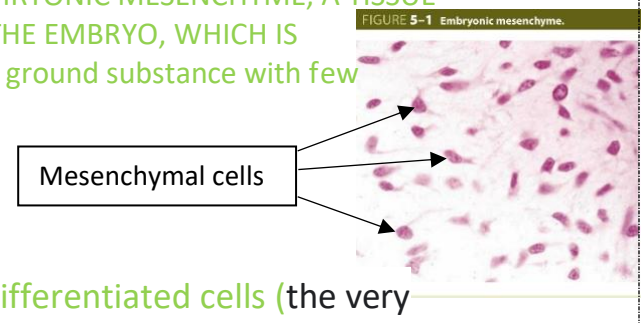




GENERAL FEATURES:

1.connective tissue originates from the mesoderm which is a layer of an embryo after fertilisation of an ovum, there is doubling and differentiation, and specialising in certain types of tissue This stage is called Trilaminar; producing 3 layers (tri); mesoderm, endoderm, and ectoderm. Each layer will give a certain type of tissue, for example mesoderm gives a connective tissue (special and general) to the organs except some parts of head and neck, they don't originate from the mesoderm.

SO,, ALL CONNECTIVE TISSUES ORIGINATES FROM EMRYONIC MESENCHYME, A TISSUE DEVELOPING MAINLY FROM THE MIDDLE LAYER OF THE EMBRYO, WHICH IS MESODERM. Mesenchyme consists largely of viscous ground substance with few collagen fibers.



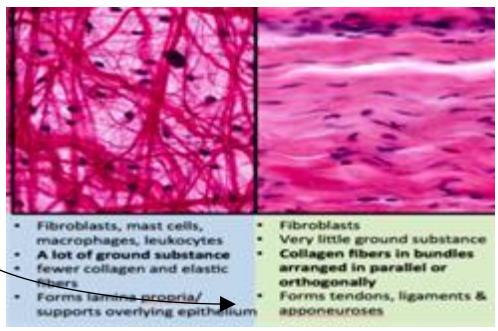
Mesenchyme consists of a population of **un**differentiated cells (the very first few **cells** in an embryo) (primitive or stem) for other tissues such as blood, the vascular endothelium, and muscle. Generally elongated but with many shapes, having large nuclei, fine (thin) chromatin and prominent (flat) nucleoli that indicate a high level of synthetic activity.

NOTE::: Stem cells are self-renewing, undifferentiated cells that divide by mitosis to produce specific body cell types. So these cells after embryogenesis, start to differentiate to fibroblast, **Osteoblasts** work in teams to build **bone, chondroblasts** work to build **cartilages,** etc. of connective tissue cells.

2.composed of cells (fixed and wandering in Type and number), fibres (elastic, collagen and Reticular fibres) and ground substance.

In this lecture and the next one we will study the proper(general).

(**What is the difference between loose and dense connective tissue proper**?)



Loose has more ground substance and less fibres

Dense has less ground substance and more fibres

3.variable vascularity.

4.variable regeneration power.

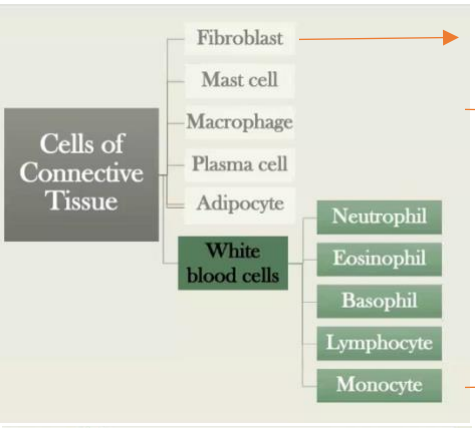
*Usually tissues that have high vascularity or receive high supply of blood they tend to have high

Loose connective tissue is the **most** common type of **connective tissue.**

regeneration power, one example is the bones (special type),is richly supplied, any fracture could be healed and fixed by the position of new bone.

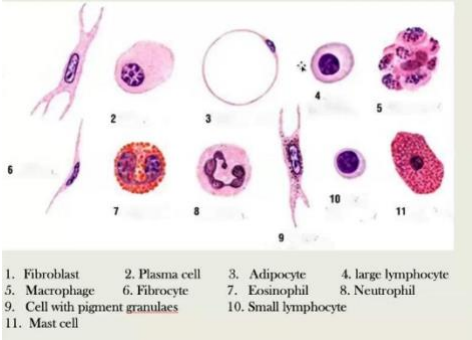
FUNCTIONS: ALL TYPES 😊

1.Structural framework (skeleton) for body. (by bones and cartilage , and both are special types).
2.Transportation of fluids and dissolved substances. (by blood (special))
3.Protection of delicate organs. (by connective tissue layers that surround the organs) if you remember the capsule around an organ it is composed of connective tissue; outside the epithelium and beneath it. So, Protection is another major function of connective tissue, in the form of fibrous capsules (1st) and bones (2nd) that protect delicate organs and, of course, the skeletal system.
4.Supports, surrounds, and connects other tissues. (we talked about it , connective tissue is under the epithelium so supports it, surrounds it, and provides it with nutrients by its vessels (because we know that epithelium is avascular) like the connection with epithelium and muscular skeleton , usually by tendons and is a strong connective tissue at the end of muscles to fix it to do its functions)
5.Storage of energy in the form of lipids. (by adipose tissue (connective tissue has fat cells that store the energy as fats or lipids)
6.Defend the body against microorganisms. (by WBCs in blood)



"The mother of the connective tissue **proper**", in special has another cells but you will see fibroblast as well. And it is responsible to build the ground substance and the fibers.

The rest of the cells are variable depending on the health state; if there is an infection you will see more plasma cells and macrophages; a strong immune reaction you will see a lot of mast cells
الخلايا الأكولة الكبيرة



Golden image
مهم حفظ الاشكال من اجل العملى(شيت 10)

White Blood Cells ((Leukocytes))

Cells of Connective Tissue

- Permanent (resident) cells دائم
  - Fibroblasts
  - Adipocytes
  - Macrophages
- Transient cells (they perform various functions in connective tissue for a short period as needed and then die by apoptosis
  - Mast cells
  - Leukocytes (white blood cells)
  - Plasma cells

ملاحظة في اخر صفحة 9

Permanent cells: that develop and remain within connective tissue, are normal components of connective tissue.
Transient cells: that migrate from the bloodstream into connective tissue in response to inflammation or tissue damage, i.e. circulate in the bloodstream and migrate into connective tissue at sites of an immune response.

That was generally, Dr. Ghada said that Fibroblasts, plasma cells, macrophages and mast cell have to be studied from the book in their details. However, WBCs you should know the main function and how they look like , but adipocytes we will discuss later on .

1.FIBROBLASTS:

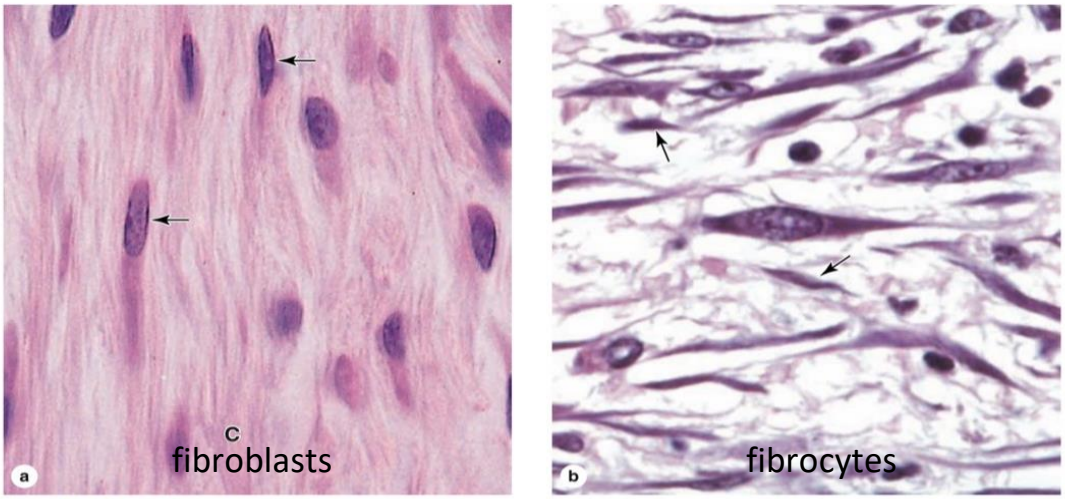
كلام كثير ...

The most common cells in connective tissue proper, produce and maintain most of the tissue's extracellular components. Fibroblasts synthesize and secrete collagen which is the only fiber can be observed with H&E stain!(the most abundant protein of the body) and elastin, which both form large fibers, as well as the GAGs, proteoglycans, and multiadhesive glycoproteins that comprise the ground substance. As described later, most of the secreted ECM components undergo further modification outside the cell before assembling as a matrix.

Distinct levels of fibroblast activity can be observed histologically (Figure 5–3b). Cells with intense synthetic activity are morphologically different from the quiescent fibroblasts that are scattered within the matrix they have already synthesized. Some histologists reserve the term "fibroblast" to denote the active cell and "fibrocyte" to denote the quiescent cell. The active fibroblast has more abundant and irregularly branched cytoplasm, containing much rough endoplasmic reticulum (RER) and a well-developed Golgi apparatus, with a large, ovoid, euchromatic nucleus and a prominent nucleolus. The quiescent cell is smaller than the active fibroblast, is usually spindle-shaped with fewer processes, much less RER, and a darker, more heterochromatic nucleus.

Figure 5-3b

FIGURE 5–3 Fibroblasts.



(a) Fibroblasts typically have large active nuclei and eosinophilic cytoplasm that tapers off in both directions along the axis of the nucleus, a morphology often referred to as "spindle-shaped." Nuclei (**arrows**) are clearly seen, but the eosinophilic cytoplasmic processes resemble the collagen bundles (**C**) that fill the ECM and are difficult to distinguish in H&E-stained sections. **(b)** Both active and quiescent fibroblasts may sometimes be distinguished, as in this section of dermis. Active fibroblasts have large, euchromatic nuclei and basophilic cytoplasm, while inactive fibroblasts (or fibrocytes) are smaller with more heterochromatic nuclei (**arrows**). The round, very basophilic round cells are in leukocytes. (Both X400; H&E)

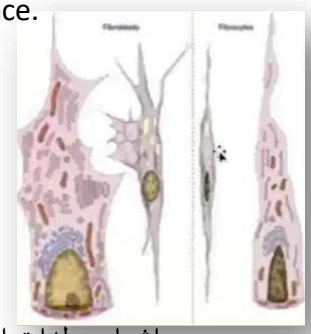
How can I differentiate between them

IMPORTANT

Fibroblasts are targets of many families of proteins called growth factors that influence cell growth and differentiation. In adults, connective tissue fibroblasts rarely undergo division. However, stimulated by locally released growth factors, cell cycling and mitotic activity resume when the tissue requires additional fibroblasts, for example, to repair a damaged organ. Fibroblasts involved in **wound healing**, sometimes called myofibroblasts, *have a well-developed contractile function and are enriched with a form of actin also found in smooth muscle cells*.

الملخص: في نوعين من الخلايا اليمفية:

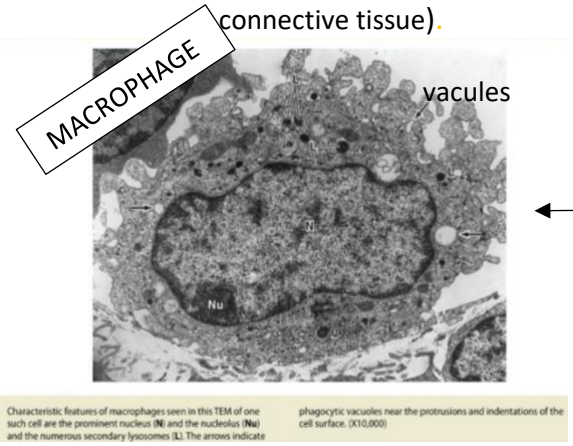
1.Fibroblast (active) because it has a large nucleus and more golgi and RER , 2. Fibrocyte (inactive) is smaller spindle shape, fewer processes, darker smaller nucleus.
Both make the extracellular fibers and ground substance.

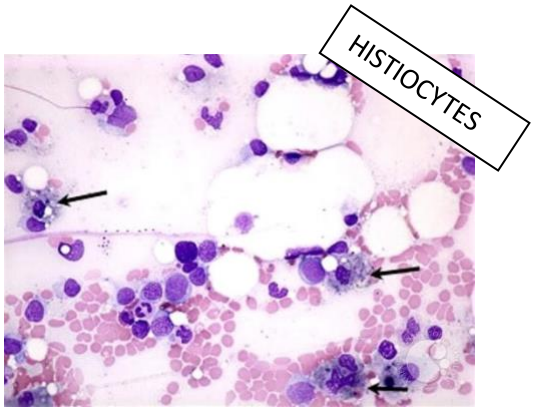


2.MACROPHAGES: الخلايا الأكولة الكبيرة

Its major function: defense by antigen presenting (اشهار مولدات الضد على سطحها) , after these cells phagocytose the pathogen, they represent their antigens by Major histocompatibility complex MHC molecules function is to determine which something is strange or not.

- Originates from a precursor: monocytes that circulate in the blood.
- Macrophages have highly developed phagocytic ability and specialize in turnover of protein fibers and removal of dead cells, tissue debris, or other particulate material, being especially abundant at sites of inflammation. Size and shape vary considerably, corresponding to their state of functional activity. A typical macrophage measures between 10 and 30 µm in diameter and has an eccentrically *away from the center* located, oval or kidney-shaped nucleus. Macrophages are present in the connective tissue of most organs and are sometimes referred to by pathologists as "**histiocytes**." Macrophages are members of the mononuclear phagocyte system, and histiocyte is a morphological term referring to tissue-resident macrophages (is also a macrophage but found usually in bone marrow, in connective tissue).





- In the TEM, macrophages are shown to have a characteristic irregular surface with pleats, protrusions, and indentations, features related to their active pinocytotic and phagocytic activities. They generally have well-developed Golgi complexes and many lysosomes.
- **Sometimes** 2 monocytes combine with each other and form a multi-nuclear macrophage.

These cells cross the epithelial wall of small venules to enter connective tissue, where they differentiate, mature, and acquire the morphologic features of phagocytic cells. Therefore, monocytes and macrophages are the same cell at different stages of maturation. Macrophages play a very important role in the early stages of (1st function) repair and inflammation after tissue damage. Under such conditions these cells accumulate in connective tissue by local proliferation of macrophages and recruitment of more monocytes from the blood (to increase the no. of macrophages). *Macrophages* are distributed throughout the body and are normally present in the *stroma of most organs*. Along with other monocyte- derived cells, they comprise a family of cells called the **mononuclear phagocyte system**. All of these macrophage-like cells are derived from monocytes, but have *different names* in *various organs*, for example, Kupffer cells in the liver, microglial cells in the central nervous system.

Langerhans cells in the skin, and osteoclasts in bone. All are long-living cells and may survive in the tissues for months. In addition to debris removal, these cells are highly important for the (2nd function) uptake, processing, and presentation of antigens for lymphocyte activation, a function discussed later with the immune system. The transformation from monocytes to macrophages in connective tissue involves increasing in cell size, increased protein synthesis, and increasing in the num- ber of Golgi complexes and lysosomes. In the TEM, macrophages are shown to have a characteris- tic irregular surface with pleats, protrusions, and indentations, features related to their active pinocytotic and phagocytic activities .They generally have well-developed Golgi complexes and many lysosomes.

First line fighters

monocytes are in the blood when they go to connective tissue their name become macrophages

TABLE 5–2 Distribution and main functions of the cells of the mononuclear phagocyte system.

Cell Type	Major Location	Main Function
Monocyte	Blood	Precursor of macrophages
Macrophage	Connective tissue, lymphoid organs, lungs, bone marrow, pleural and peritoneal cavities	Production of cytokines, chemotactic factors, and several other molecules that participate in inflammation (defense), antigen processing, and presentation
Kupffer cell	Liver (perisinusoidal)	Same as macrophages
Microglial cell	Central nervous system	Same as macrophages
Langerhans cell	Epidermis of skin	Antigen processing and presentation
Dendritic cell	Lymph nodes, spleen	Antigen processing and presentation
Osteoclast (from fusion of several macrophages)	Bone	Localized digestion of bone matrix
Multinuclear giant cell (several fused macrophages)	In connective tissue under various pathological conditions	Segregation and digestion of foreign bodies

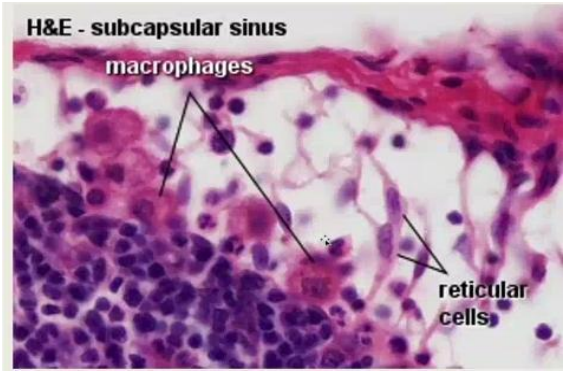
Multi-nucleated
Suggested:
https://youtu.be/0dV1Bwe2v6c

All are from monocytes but in different organs with a major function: phagocytosis, antigen presenting, secretion of cytokines

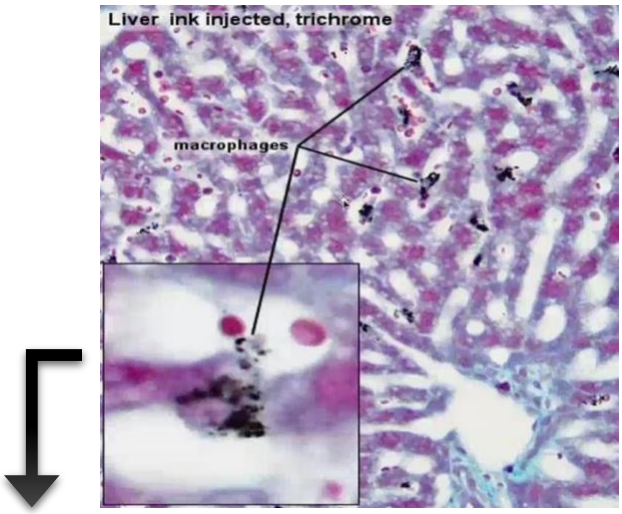
There are Langerhans in the epidermis of skin are Langerhans cells , MACROPHAGES.
There are Langerhans in pancreas are Langerhans islets.

NOTE (to deep understand only):: there are two types of macrophages :1. A resident species, which has formed during embryonic life and has a lifespan of several years, and the atmosphere

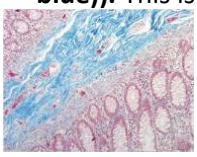
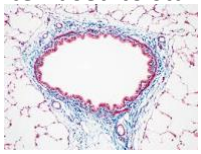
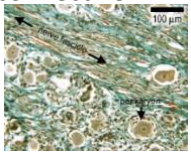
of the tissue decays as it moves. 2.In a transient species, they are produced by the bone marrow periodically, and their life span is short.



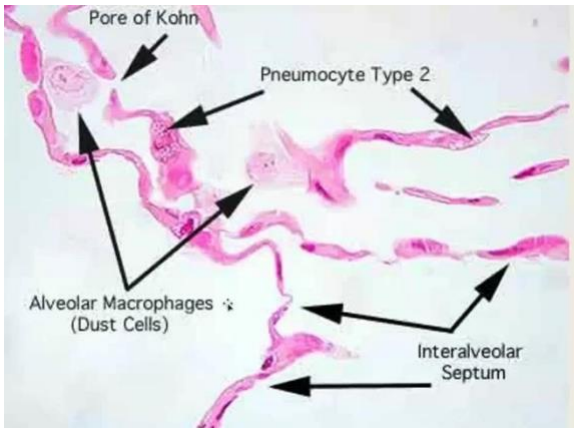
- H&E : because it is stained pink and blue, bright field microscope.
- No. 5 in golden image, large cells with a large nuclei



- Kupffer cells(liver)
- The stain: NOT H&E , because the cytoplasm is blue and nuclei are pink! It is Masson's trichrome, is a three-colour staining protocol used in histology. ((Nuclei and other basophilic (basic-liking) structures are stained **blue**, cytoplasm, muscle, erythrocytes and keratin are stained bright-**red**. Collagen is stained **green or blue)).** This is often used to stain connective

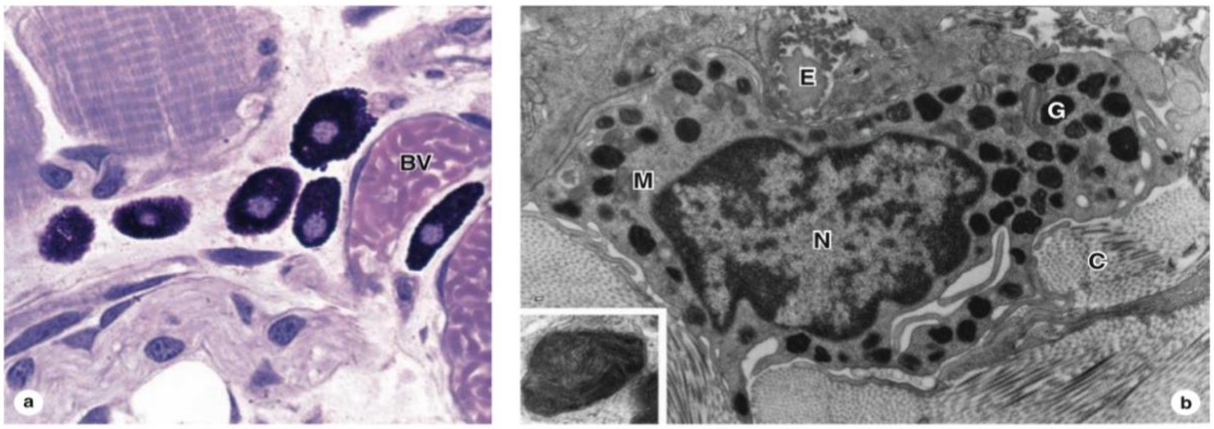
This image is made with 2 steps: the liver was injected with ink flow in blood stream and engulfed by macrophages , then stained with fluorescence, another way of staining is immunohistochemistry (antibodies to show macrophages(ink))



- ANOTHER TYPE OF MACROPHAGES (NOT INCLUEDE IN THE PREVIOS TABLE) but you should know it: ALVEOLAR macrophages (Dust Cells) . **Alveolar macrophages** are mononuclear phagocytes found in the **alveoli** of the lungs. They ingest small inhaled particles resulting in the degradation, clearance and presentation of the antigen .

3.MAST CELLS:

FIGURE **5–5** **Mast cells.**



Mast cells are components of loose connective tissues, often located near small blood vessels (**BV**). **(a)** They are typically oval shaped, with cytoplasm filled with strongly basophilic granules. (X400; PT)

(b) Ultrastructurally mast cells show little else around the nucleus (**N**) besides these cytoplasmic granules (**G**), except for occasional mitochondria (**M**). The granule staining in the TEM is heterogeneous and variable in mast cells from different tissues; at higher magnifications some granules may show a characteristic scroll-like substructure (inset) that contains preformed mediators such as histamine and proteoglycans. The ECM near this mast cell includes elastic fibers (**E**) and bundles of collagen fibers (**C**).

>Mast cells are oval or irregularly shaped cells of connective tissue between 7 and 20 um in diameter. They are filled with basophilic secretory **granules** (اوضح دليل لتمييزها) which often obscure the central nucleus.

> These granules are electron-dense and of variable size :0.3 to 2.0 um in diameter.

> Mast cell granules display metachromasia, which means that they can change the color of some basic dyes (eg, toluidine blue) from blue to purple or red.

> Mast cells function in the localized release of many bioactive substances important in the local inflammatory response

> The main molecules they release inside granules are heparin (an anticoagulant) and histamine (increases vascular permeability)

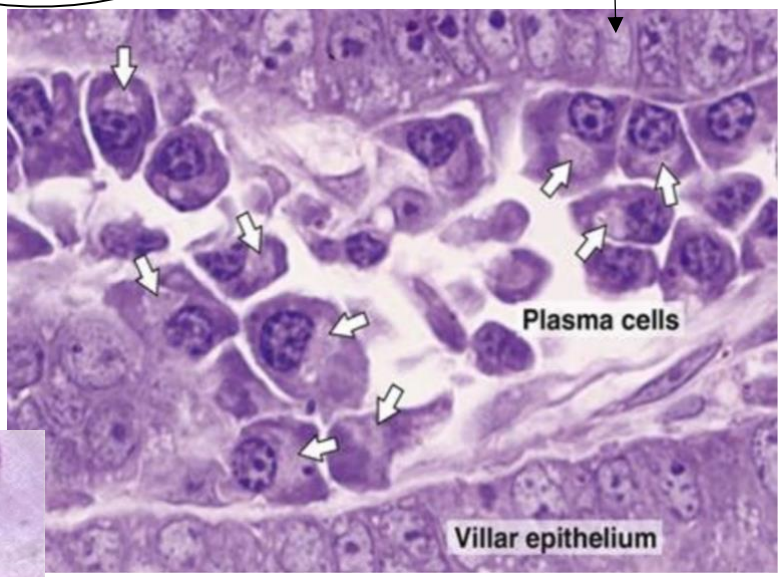
Because of the high content of acidic radicals in their sulfated GAGs, **mast cell *granules* display metachromasia** (the ability to change its color), which means that they can change the color of some basic dyes (eg, toluidine blue) from blue(as It should) to purple or red. The granules are poorly preserved by common fixatives, so that mast cells may be difficult to identify in routinely prepared slides.

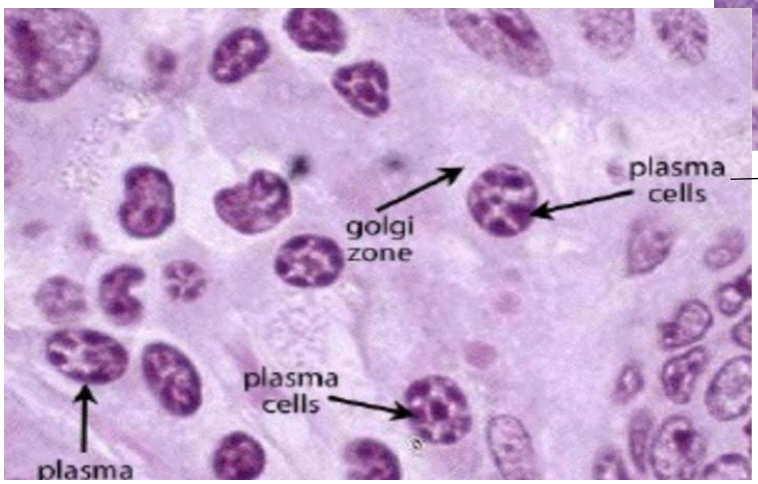
They are involved in allergic reactions known as **immediate hypersensitivity reactions**.(تفاعلات فرط الحساسية).

4.PLASMA CELLS:

Plasma cells are lymphocyte-derived, antibody-producing cells. They are relatively large ovoid cells with basophilic cytoplasm rich in RER and a large Golgi apparatus near the nucleus that may appear pale in routine histologic preparations (negative Golgi staining). The nucleus of the plasma cell is generally spherical eccentrically placed. Many of these nuclei contain compact, peripheral regions of heterochromatin alternating with lighter areas of euchromatin (clock-face or cart-wheel appearance). Their average lifespan is only 10-20 days.

- You could see them or not , but you will see a lot of them in inflammation, because its function in antibody production and secretion.
- Precursor: **B** lymphocytes.
- Relatively big in size, as well as its nucleus; you can easily distinguish it.





It has an obvious distinguishable nucleus and clumping chromatin. But here I can't distinguish between nucleus and chromatin.

Connective Tissue Fibers

Collagen	**Undulating course of longitudinally striated bundles, form meshwork of variable texture, stain pink-red in H&E. Nonextensile.**
Elastic	**Forms sheets or lamina, Unstained in H & E. Reversibly extensible. Stains brown-black in Orcein, Resorscin Fuchsin, and Verhoeff-van Gieson's**
Reticular	**Delicate network, Unstained in H & E. Reversibly extensible. PAS +ve, stains black in $AgNO_3$ (Argyrophilic).**

- In dense connective tissue, you could see more collagen fibers, particularly, ***type 1***.
- Liver and lymph nodes; the 2 places where you can see the reticular fibers.
- STAINING: collagen ⟶ can be seen with H&E(positively stained by H&E) , but if you want to see the amount (special stain).
  Elastic+ reticular ⟶ cant be seen with H&E(negatively stained by H&E) , (have to stain with a special one)
- Extensible means strectable(it's length can be increased and adjusted), in contrast nonextensile means it's length can't be increased
- We will see all of them under microscope in sheet no. 10.

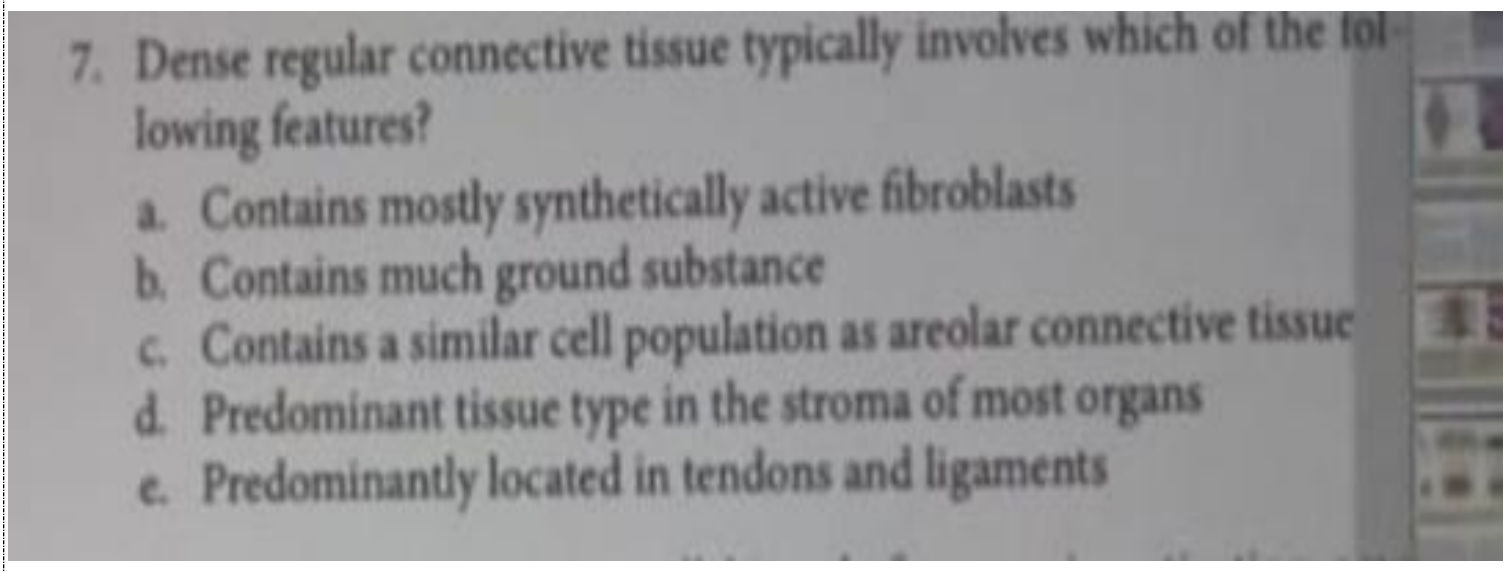
Flash cards on this chapter 😊
https://quizlet.com/387003333/connective-tissue-flash-cards/

1. Which of the following connective tissue components is located in the ECM but not in the ground substance?
   a. Collagen bundles
   b. Fibronectin
   c. GAGs
   d. Hyaluronan
   e. Proteoglycans

2. What cells numerous in loose connective tissue are filled with secretory granules and stain with metachromasia?
   a. Macrophages
   b. Mast cells
   c. Fibrocytes
   d. Active fibroblasts
   e. Leukocytes

4. What is an important part of the role played by macrophages during maintenance and renewal of strong extracellular fibers in connective tissue?
   a. Storage for a major energy source needed for ECM maintenance
   b. Production of specific collagen subunits
   c. A sentinel function against invaders entering the ECM
   d. Secretion of matrix metalloproteinases
   e. Presentation of antigens important for assembly of collagen bundles

5. Sulfated GAGs are important constituents of what extracellular structures?
   a. Hyaluronan
   b. Elastic fibers
   c. Type I collagen
   d. Proteoglycans
   e. Multiadhesive glycoproteins



Answers(respectively) ::a, b, d, d, e

THAAAANK YOUUU<3<3