Topic 4. Connective tissue proper (fibrous connective tissue)

Control questions

1. General characteristic and functions of connective tissues.
2. Connective tissue proper: classification, structural characteristic, distribution and functions.
3. Loose connective tissue. Fixed and migratory cells: origination, structural and functional characteristic.
4. Extracellular matrix – types of fibers and ground substance: formation, structure and function.
5. Dense regular and irregular connective tissue.
6. Connective tissues with special properties: adipose, reticular and elastic.
7. Embryonic connective tissues: mesenchymal and mucous.

Question 1. General characteristic and functions of connective tissues.

General characteristic and functions of connective tissues are:

1. Mesenchymal origin;
2. Extensive intercellular substance called an extracellular matrix (ECM), predominating over the cells
3. Participation in support of organism homeostasis.

Question 2. Connective tissue proper: classification, structural characteristic, distribution and functions.

Fibrous connective tissue has general characteristic of another connective tissues. Similar to other tissues it consists of 2 components - cells and extensive intercellular substance. The intercellular substance of connective tissue proper consists of ground substance and protein fibers: collagenous, reticular and elastic.

There are several types of fibrous connective tissue according to the types, amount and arrangement of fibers. There are two main types: loose and dense connective tissues.

Dense connective tissue differs from the loose one mainly in the great predominance of the fibers over the little ground substance and cellular component. Dense connective tissue is classified into two basic types based on the organization of its collagen fibers: dense irregular connective tissue and dense regular connective tissue.

Dense irregular connective tissue contains mostly collagen fibers that are arranged in bundles, lying in various directions. Because of its high proportion of collagen fibers, dense irregular connective tissue provides significant strength. Dense irregular connective tissue is represent in the reticular layer (or deep layer) of skin dermis and in some capsules of inner organs.

In dense regular connective tissue, the predominant fibers are arranged in parallel array and are densely packed to provide maximum strength. Dense regular connective tissue is the main functional component of tendons, ligaments, and aponeuroses.

Main function of the dense connective tissue is mechanical support.

Loose (areolar) connective tissue has different types of cells and all types of fibers, which are arranged in different directions, forming a network and great ground substance predominating over the fibers.

Loose connective tissue is widely distributed and the most abundant in the human body because it forms the stroma and capsules of inner organs, where it is located beneath the epithelia and accompanies, and surrounds the vessels and nerves.

Functions of loose connective tissue are:

1. Participation in the homeostasis, because it provides the exchange of metabolites between blood and all tissues and plays a significant role in the nutrition of the other tissues.
2. Participation in the suspension and support for the other tissues.
3. Protection against infections by the phagocytosis and production of antibodies.
4. Plastic function (repairing after injury).

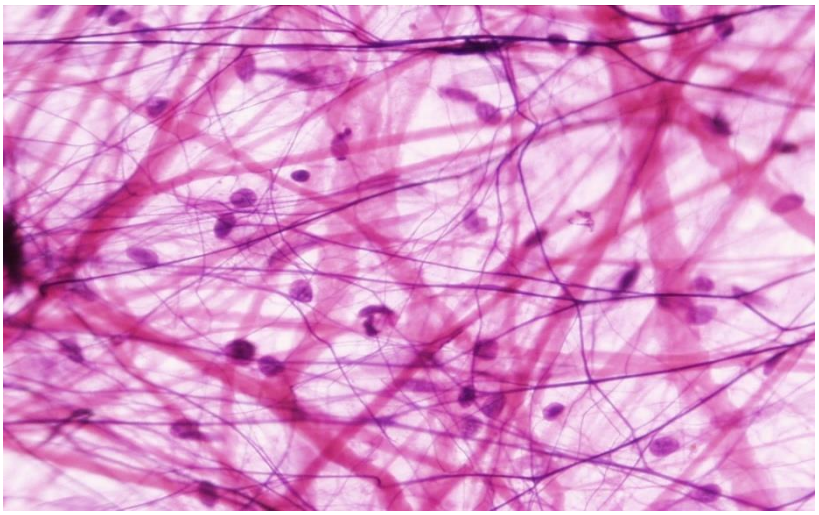


Figure 4.1. Photomicrograph of loose connective tissue.

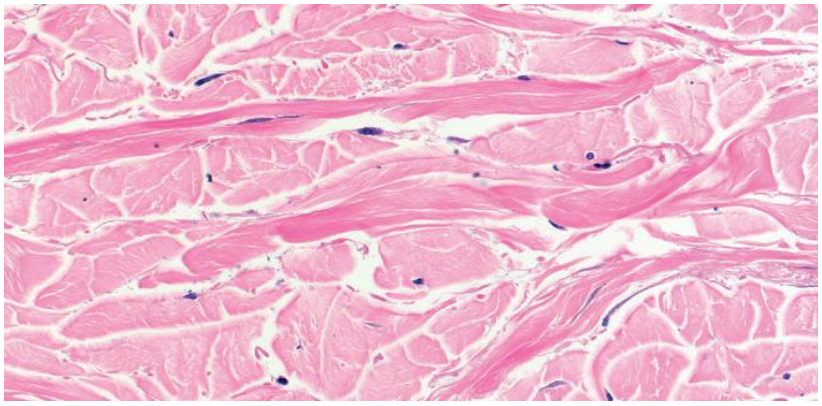


Figure 4.2. Photomicrograph of dense irregular connective tissue [14]**.**

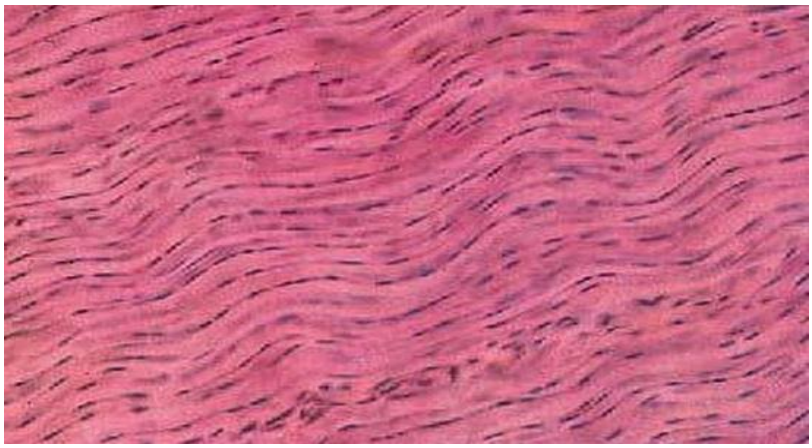


Figure 4.3. Photomicrograph of dense regular connective tissue.

Question 3. Loose connective tissue. Fixed and migratory cells: origination, structural and functional characteristic.

There are two groups of the loose connective tissue cells: 1) a relatively stable population of fixed or intrinsic cells and 2) a population of migratory, or extrinsic cells.

Fixed cells are fibroblasts, fibrocytes, myofibroblasts, adipose (fat) cells, reticular cells, pericytes, adventitial (perivascular) cells and pigment cells.

All fixed cells except only pigment have mesenchymal origin and have a common cell precursor - mesenchymal stem cells called also adventitial cell. Adventitial cells are perivascular cells, because

found around capillaries and venules. Adventitial cells are undifferentiated cells that give rise to differentiated cells that function in the repair and formation of new tissue for wound healing and participate in the blood vessels regeneration.

A population of migratory or extrinsic cells have mesenchymal origin too. Migratory cells enter the loose connective tissue from blood and they are developed from HSC of bone marrow. Extrinsic cells are macrophages, plasma cells, all types of T-lymphocytes, neutrophils, eosinophils, basophils and mast cells [3].

Pigment cells have a neural crest origin. Pigment cells are irregular shape, containing ovoid light nucleus and inclusions of pigment melanin in their cytoplasm.

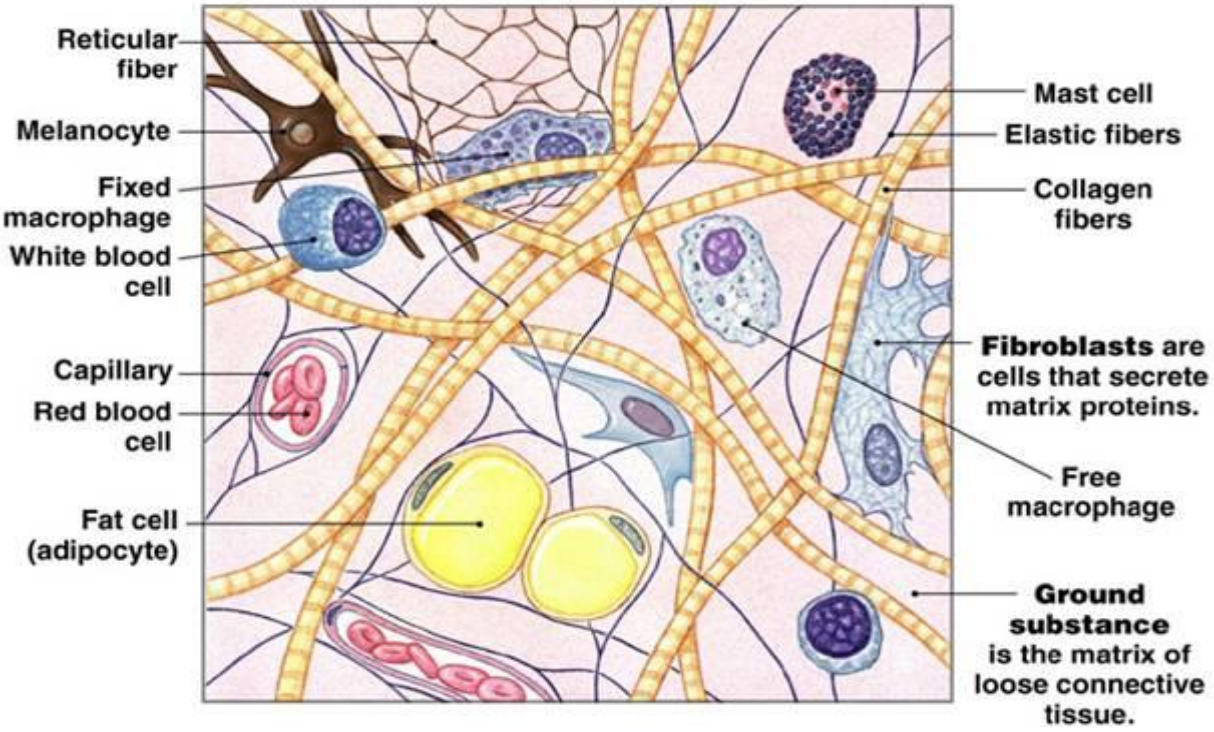


Figure 4.4. Diagram of loose connective tissue components.

Fibroblast

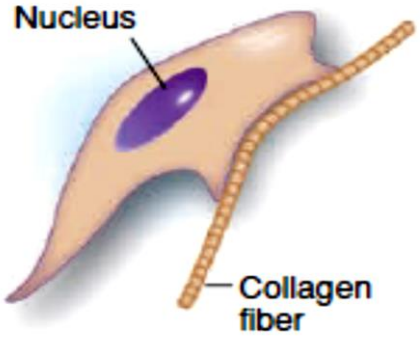


Figure 4.5. Diagram of fibroblast [1].

Fibroblasts are the most numerous cells, which elaborate all components of extracellular substance (fibers, and carbohydrates of the ground substance).

Fibroblast is a stellate cell with several processes. Its cytoplasm contains well developed apparatus of the synthesis: rER and Golgi complex. Nucleus of fibroblast is ovoid, large, and pale-stained containing 1 or 2 nucleoli.

In adults, fibroblasts rarely undergo division. Mitoses are observed only when connective tissue is damaged.

Functions of fibroblast:

1) Formation, maintaining, and reorganization of the extracellular matrix;
2) Regulatory.

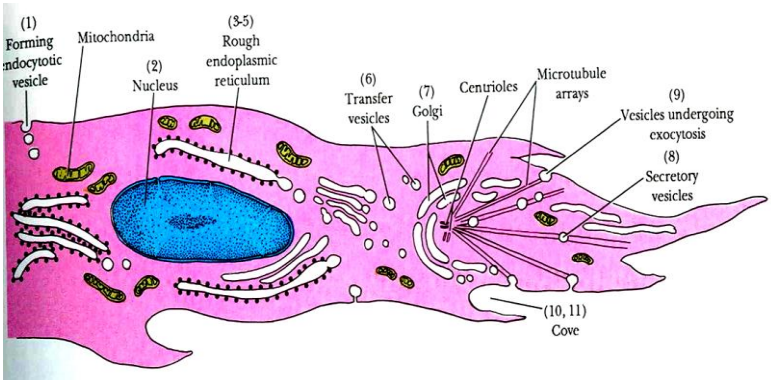


Figure 4.6. Diagram of fibroblast's ultrastructure [12].

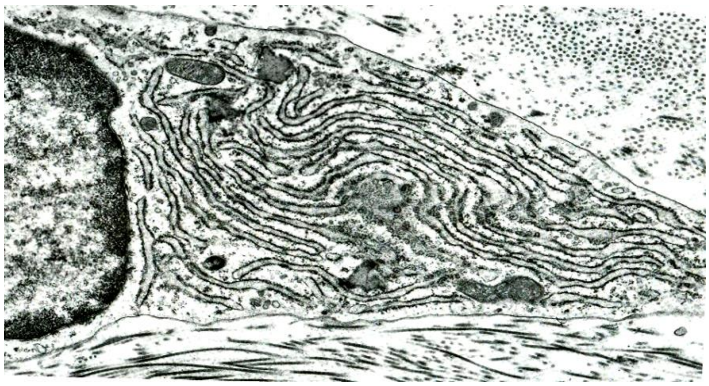


Figure 4.7. Electron micrograph of fibroblast [15].

Fibrocyte

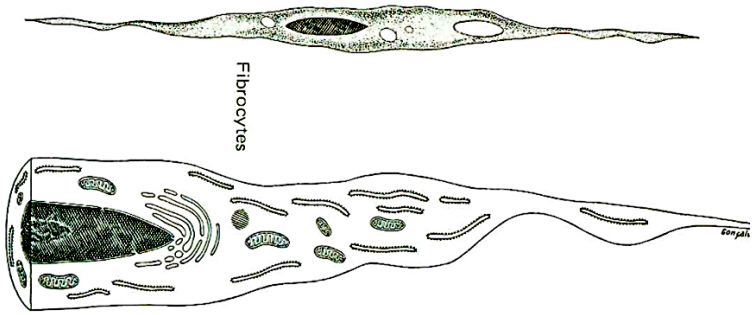


Figure 4.8. Diagram of fibrocyte [7].

Some fibroblasts transform into the fibrocytes. Thus, the fibrocytes are mature old form of fibroblast. They are spindle-shaped cells with smaller, darker, elongated nucleus and an acidophilic cytoplasm. Fibrocytes can not produce fibers, but they produce regulatory factors for the stability of the extracellular substance.

Myofibroblast

Myofibroblasts combine properties of both the fibroblast and the smooth muscle cell. They are characterized by the presence of bundles of actin filament with associated myosin proteins. In repairing tissue myofibroblasts help to form the collagen and retraction and shrinkage of scar tissue.

Connective tissue macrophages (histiocytes)

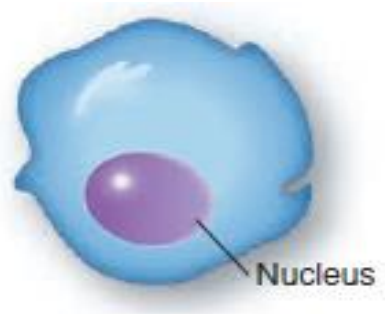


Figure 4.9. Diagram of histiocyte's ultrastructure [1].

Connective tissue macrophages, also known as tissue histiocytes are derived from blood monocytes. Monocytes migrate from the bloodstream into the connective tissue, where they differentiate into macrophages. The surface of histiocyte has numerous folds and projections. The surface folds engulf the substances to be phagocytosed. Histiocytes contain an oval or kidney-shaped nucleus located eccentrically and a large Golgi apparatus, rER, mitochondria and abundant of lysosomes.

Functions of histiocytes:

1) Phagocytosis, either as a nonspecific defense activity (as in phagocytosis of bacteria) or as a clean up operation (as in phagocytosis of cell debris);
2) Participation in the immune response as APC (antigen presenting cells);
3) Regulatory.

In pathologic conditions, histiocytes may jncrease in size, forming epithelioid cells, or several histiocytes may fuse to form multinuclear giant cells [5].

Mast cells

Mast cells are large, ovoid, connective tissue cells (20 to 30 μm in diameter) with a spherical nucleus and cytoplasm filled with large, intensely membrane-limited basophilic granules. The mast cells are related, but not identical to the basophils of the blood, that contain similar large intensely basophilical staining granules in cytoplasm.

The surface of mast cells contains specific receptors for IgE, which are produced by plasma cells. Most IgE molecules are fixed on the surface of mast cells and blood basophils. Binding of specific antigen to exposed IgE molecules on the mast cell surface leads to mast cell activation, which results in granule exocytosis (degranulation) and the release of granules content into the extracellular matrix.

Thus, mediators release during mast cell activation, as a result of interactions mast cells with allergens. It leads to variety symptoms characteric for allergic reactions down to anaphylactic shock as a potentially fatal condition. These allergic reactions known as "immediate hypersensitivity reactions" because they occur in a few minutes after penetration by antigen.

The content of mast cells basophilic granules are chemical substances known as mediators of inflammation. They are histamine, heparin, eosinophil chemotactic factor (ECF) and neutrophil chemotactic factor (NCF); slow-reacting substance of anaphylaxis (SRS-A).

Histamine is a biogenic amine that increases the permeability of small blood vessels, causing edema in the surrounding tissue and a skin reaction demonstrated by an itching sensation. It increases mucus production in the bronchial tree and promotes contraction of smooth muscle in the pulmonary airways.

Heparin is a sulfated GAG that is an anticoagulant.

Eosinophil chemotactic factor (ECF) and neutrophil chemotactic factor (NCF) attract eosinophils and neutrophils to the site of inflammation. The secretions of eosinophils counteract the effects of the histamine and leukotriens.

Slow-reacting substance of anaphylaxis (SRS-A) is leukotriene, which is modified lipids. Leukotrienes trigger prolonged constriction of smooth muscle in the pulmonary airways, causing bronchospasm [13].

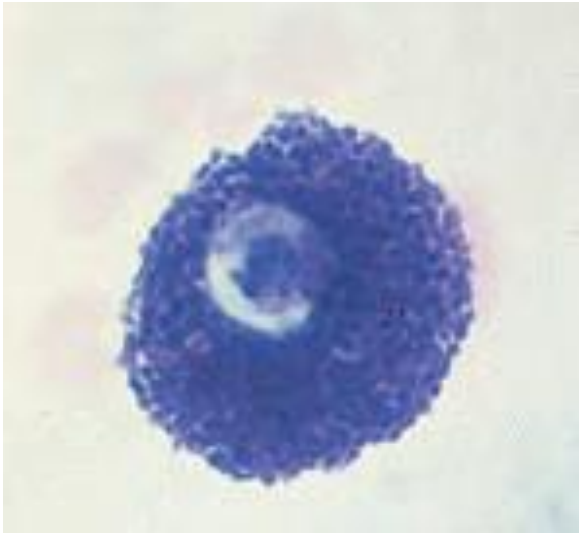


Figure 4.10. Photomicrograph of mast cell [13].

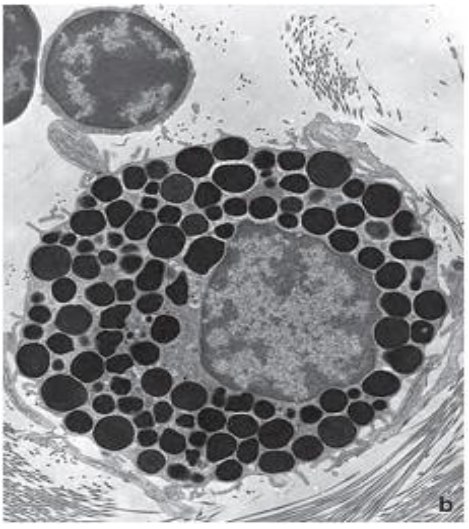


Figure 4.11. Electron micrograph of mast cell [13].

Plasma cells

Plasma cells are antibody-producing cells derived from B-lymphocytes for their antigen depending differentiation. They are effecter cells of humoral immunity.

Plasma cells are average size, ovoid or egg like cells with spherical and eccentrically placed nucleus and basophilic cytoplasm. The cytoplasm of plasma cells shows intensive basophilia because contains numerous r-EPR, providing the biosynthesis of antibodies. The juxtanuclear Golgy complex and the centrioles appear as a pale clear area near the nucleus. Clear area near the nucleus is evidence

morphological feature for diagnostic of plasma cells. The nucleus of plasma cells contains large clumps of heterochromatin alternating with clear areas of euchromatin. This arrangement has been described as analog clock-face [13].

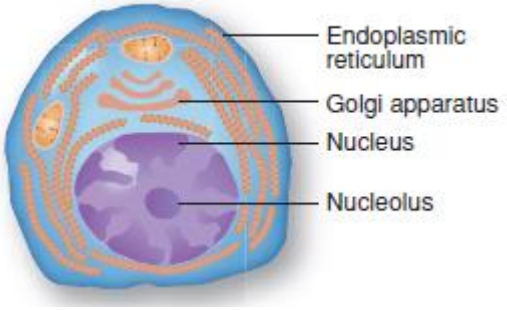


Figure 4.12. Diagram of plasma cell's ultrastructure [1].

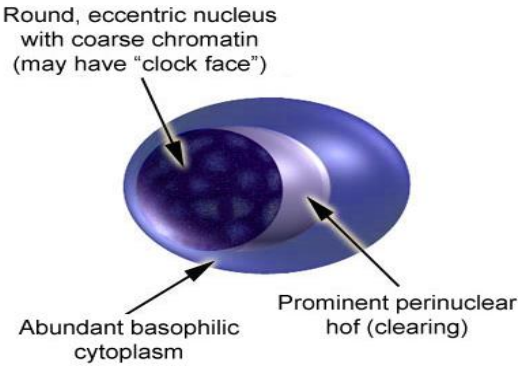


Figure 4.13. Diagram of plasma cell

Question 4. Extracellular matrix – types of fibers and ground substance: formation, structure and function.

Extracellular matrix (ECM)

Fibers:
1. **Collagenous**
2. **Reticular**
3. **Elastic**

Ground amorphous substance:
1. **Glycosaminoglycans (GAGs)**
2. **Proteoglycanes**
3. **Structual glycoproteines**

Functions of the extracellular substances:
1. Structural and mechanical;
2. Integrative;
3. Microenvironment for the cells;
4. Selective barrier;
5. Regulatory.

Collagen fiber: formation, structure and function.

Collagen fiber formation involves events that occur both within and then outside the fibroblast.

I. **Intracellular events** include:

1) Collagen α chains are synthesized in the rER of fibroblast. They are pro–α chains called preprocollagen molecules;

2) In rER a triple helix is formed by three α chains, except at the terminals where the polypeptide chains remain uncoiled. The resultant molecule is procollagen molecule;

3) The procollagen molecules pass to the Golgi apparatus where they are packaged into secretory vesicles which transported to the cell surface to realize from the fibroblast by exsocytosis.

II. Formation of collagen fibrils or fibrillogenesis involves **extracellular events**, which are:

4) Secretion of procollagen from the cell, during which the enzyme procollagen peptidase associated with the fibroblast cell membrane, will be cleave the uncoiled ends of the procollagen molecule with formation of mature collagen molecule, also called tropocollagen;

5) The aggregated collagen molecules that align in rows and self-assemble longitudinally in head-to-tail fashion and cross-linked by covalent bonds to form the final collagen fibrils.

6) Collagen fiber is a bundle of collagen fibrils.

Collagen fibrils have closely spaced transverse bands that repeat every 68 nm along the length of the fibril. This banding pattern depends on the size and shape of the collagen molecule and the arrangement of the molecules within fibril [13].

Collagenous fibers are thick, slightly wavy. They are present in all types of the connective tissue and are responsible for the tissue tensile strength.

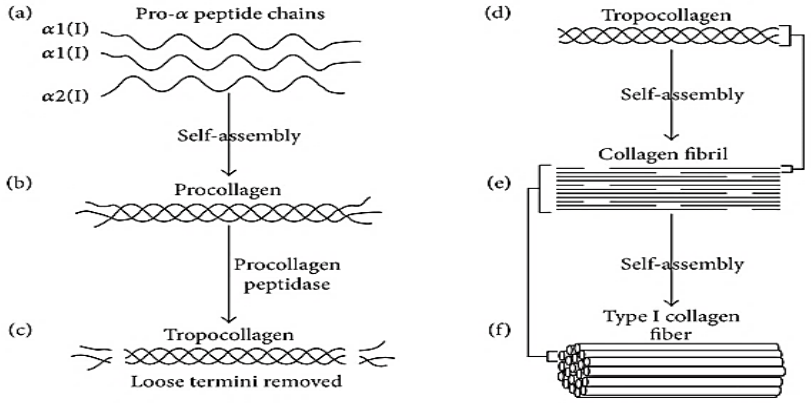


Figure 4.14. Diagram of collagen fiber formation.

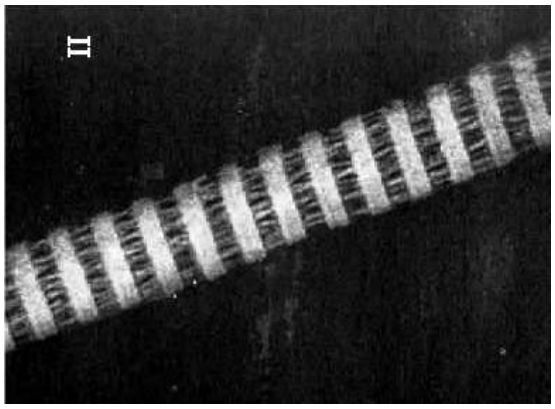


Figure 4.15. Electron micrograph of collagen fibril [15].

Elastic fiber: formation, structure and function.

Elastic fibers are smaller, thinner and less variable in size than collagen fibers and they branch and anastomose to form a network. The elastic fibers can stretch and relax.

Elastic fibers have two components - amorphous central component presenting by elastin (protein) and microfibriles, aggregated in bundles round the elastin. Protein of the microfibrils is fibrillin.

Fibroblasts synthesize the elastic fibers also. They secrete elastin molecules which joined by covalent bonding to form a crosslinked network [13].

Ground substance

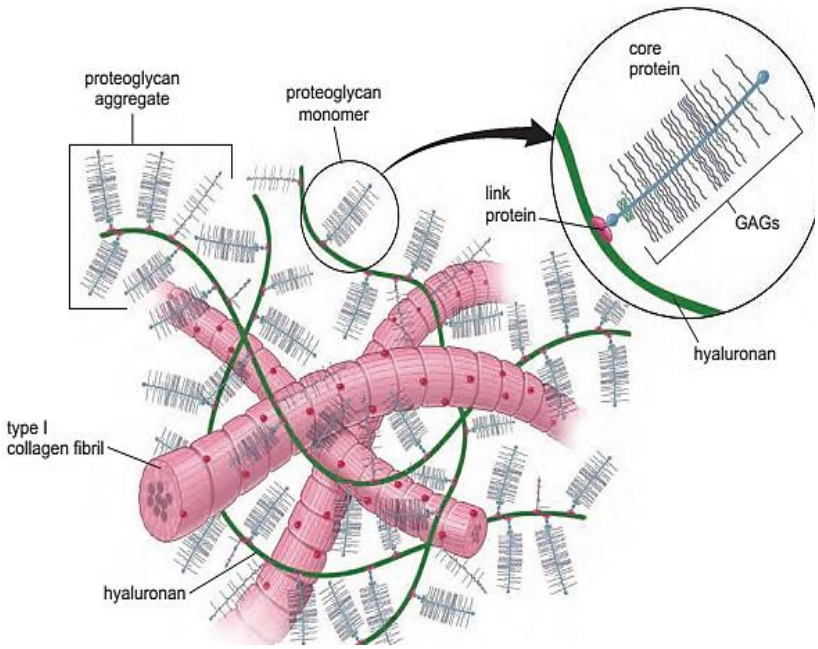


Figure 4.16. Diagram of proteoglycan structure [13].

Ground substance is a part of the extracellular matrix that occupies the spaces between the cells and fibers. It consists of:

1) Glycosaminoglycans (GAGs) - hyaluronic acid or hyaluronan and different sulfates: keratan-sulfate, chondroitin-sulfate, dermatan sulfates and so on.

2) Proteoglycans - GAGs joined to the core protein.

3) Multiadhesive glycoproteins - fibronectin and laminin.

The main function of glycosaminoglycans is structural and interaction with collagen fibrils. Multiadhesive glycoproteins play an important role in stabilizing the extracellular matrix and linking it to the cell surfaces [13].

Loose connective tissue (slide)

Stain: iron hematoxylin

Using this illustration you must perform the exercise 2 of album (topic " Connective tissue proper")

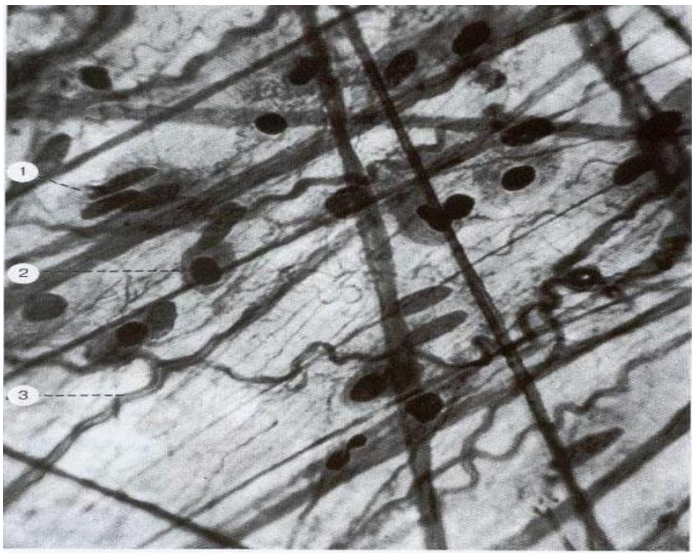


Figure 4.17. Photomicrograph of loose connective tissue.

1. **Fibroblast**
2. **Histiocyte**
3. **Fibers**

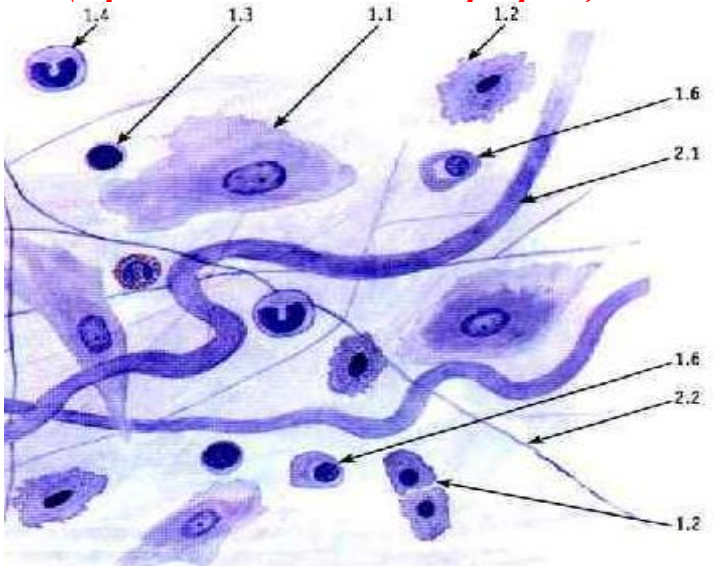


Figure 4.18. Diagram of loose connective tissue [18].

- **1.1. Fibroblast**
- **1.2. Histiocyte**
- **1.3. Lymphocyte**
- **1.4. Monocyte**
- **1.6. Plasma cell**
- **2.1. Collagen fiber**
- **2.2. Elastic fiber**

Question 5. Dense regular and irregular connective tissue. Human finger skin (slide)

Stain: hematoxylin-eosin

Using this illustration you must perform the exercise 10 of album (topic " Connective tissue proper")

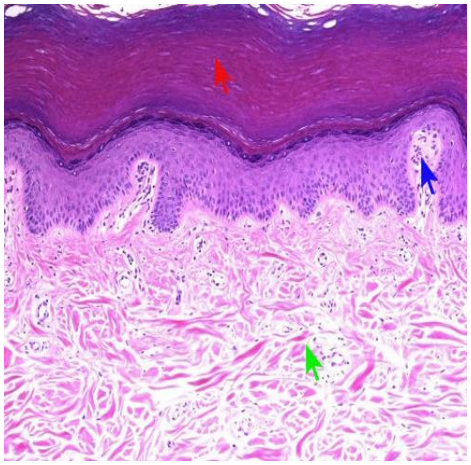


Figure 4.19. Photomicrograph of finger skin.

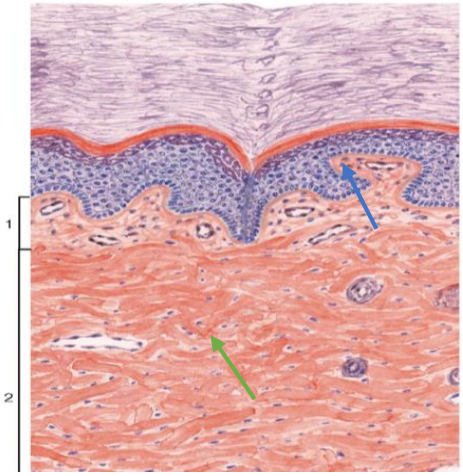


Figure 4.20. Diagram of finger skin [18].

1. Papillary layer of dermis is represented by loose connective tissue
2. Reticular layer of dermis is represented by dense irregular connective tissue

Dense regular connective tissue

In tendons as well as ligaments, the collagen fibers are very densely packed and are organized in parallel array into fascicles. Rows of fibroblasts situate between fibers. In tendons this cells are called tendinocytes. The fascicles are separated from one another by thin layers of loose connective tissue with vessels and nerves called endotendineum. The fascicles may be grouped into larger functional units by a thicker, surrounding connective tissue called peritendineum. Finally, the tendons and ligaments are surrounded by dense irregular connective tissue called epitendineum.

Tendon (slide)

Stain: hematoxylin-eosin

Using this illustration you must perform the exercise 11 of album (topic "Connective tissue proper")

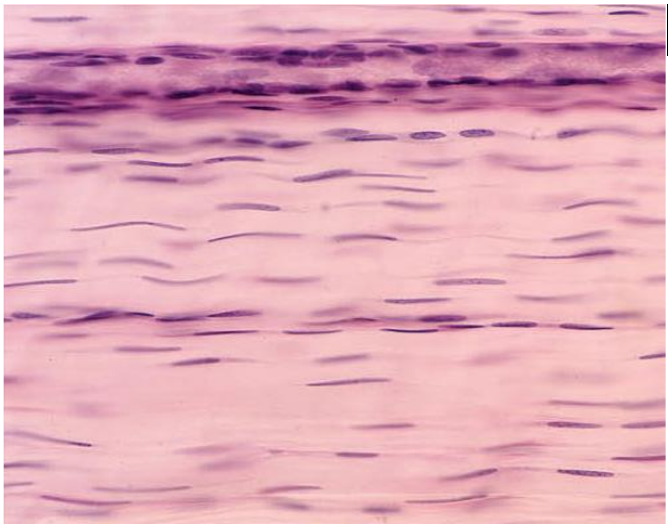


Figure 4.21. Photomicrograph of tendon in longitudinal section [13].

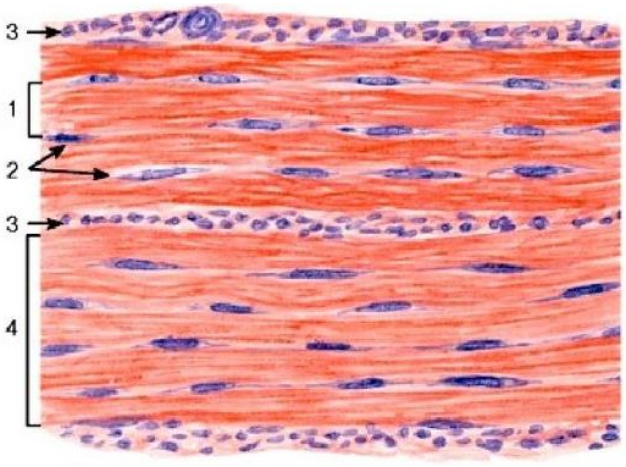


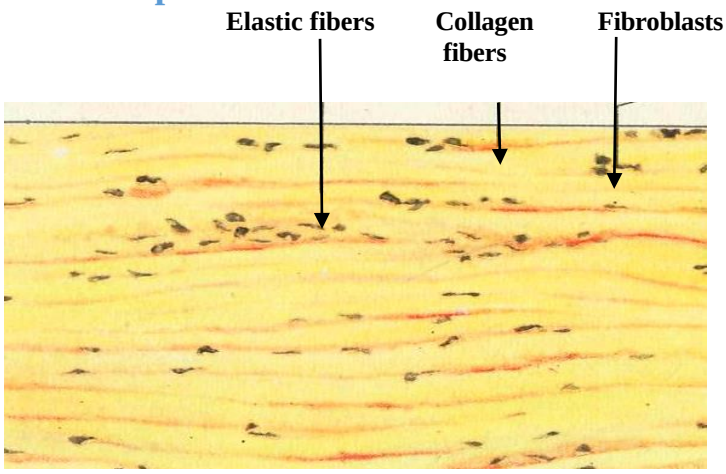
Figure 4.22. Diagram of finger skin [18].

1. Collagen fibers
2. Tendinocytes
3. Endotendineum
4. Fascicle

Elastic ligament (slide)

Draw this slide in exercise 12 of album (topic " Connective tissue proper")

Stain: picrofuchsin



Elastic fibers are stained with yellow color by picrofuchsin

Figure 4.23. Diagram of elastic ligament longitudinal section [15].

Question 6. Connective tissues with special properties: adipose, reticular and elastic.

Individual fat cells or adipocytes, and small groups of adipocytes are found throughout loose connective tissue. However the tissues in which adipocytes are the primary cell type are designated adipose tissue. Adipose tissue is a specialized connective tissue.

There are two types of adipose tissue: white (unilocular) and brown (multilocular). White adipose tissue is the predominant type in adult humans. Brown adipose tissue is present in humans during fetal life but diminishes during the first decade after birth.

Unilocular adipocytes are large cells, sometimes 100 μm or more in diameter. When isolated, white adipocytesare spherical, but they may appear polyhedral or oval in groups of cells. The mature unilocular adipocyte is characterized by a single, large lipid droplet surrounded by a thin rim of cytoplasm. The nucleus is flattened and displaced to one pole of cell. Because these cells have a single lipid droplet, they are designated unilocular adipocytes or mature lipocytes.

In routine histologic sections, the lipid is lost through extraction by organic solvents. Therefore, in preparations the adipose tissue appears as a delicate meshwork of polygonal profiles.

In newborns, brown adipose tissue makes up about 5% of the total body mass. In newborns, the brown adipose tissue helps to offset the extensive heat loss that results high surface-to-mass ratio and to avoid lethal hypothermia. Thus, heat production (thermogenesis) is main function of brown adipose tissue.

In adults, some amount of brown adipose tissue there is around the kidney and adrenal glands, in regions of the neck and back and in mediastinum.

The cells of brown (multilocular) adipose tissue are smaller than those of white adipose tissue. The nucleus of a mature multilocular adipocyte is in a central position and spherical shape. The cells contain the numerous fat droplets of varying size. The multilocular adipocyte contains numerous mitochondria with large amounts of cytochrome oxidase, which imparts the brown color to the cells **[13].**

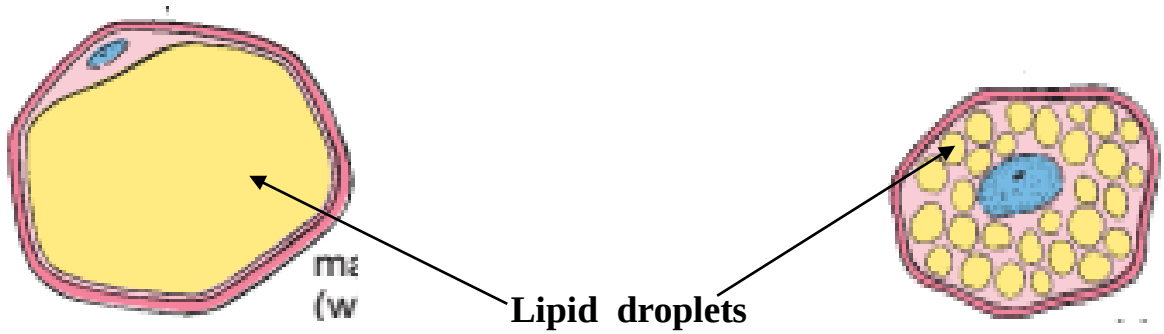


Figure 4.24. Diagram of white fat cell [13].

Figure 4.25. Diagram of brown fat cell [13].

Functions of fat cells:

1) Energy storage;
2) Hormones production;
3) Source of metabolic water;
4) Nutritive function;
5) Mechanical support;
6) Heat production (thermogenesis).