



Histology

faculty of medicine - JU2017

Sheet

Slides

Number

7

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Connective Tissue

☆Features of the connective tissue :

- Composed of cells, fibers and extracellular matrix.
- Highly vascular.
- Variable regenerative power.
- Originates from the mesenchyme.

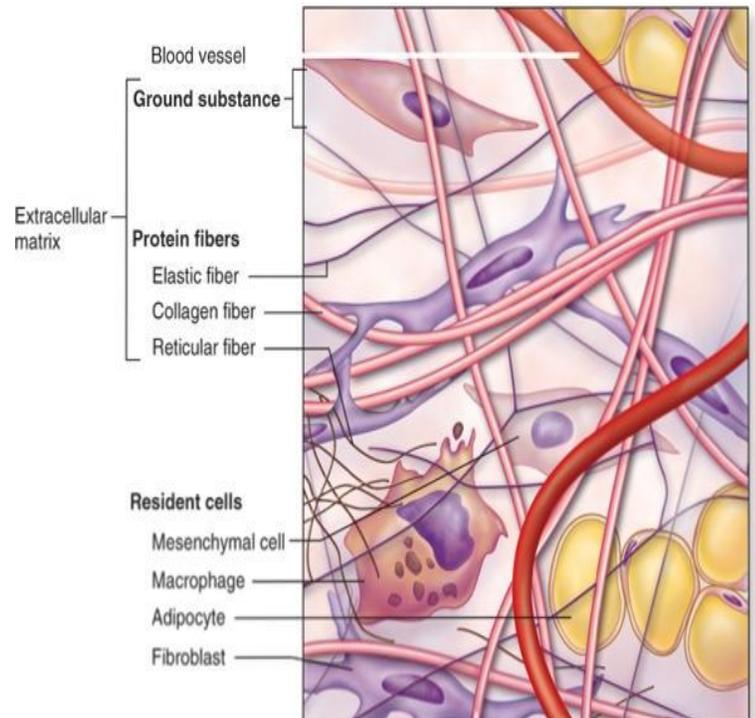
☆Functions of Connective Tissue :

Support, Defense and protection, Storage, and Transport (for gases, certain molecules and waste products).

☆Extracellular matrix :

Composed of ground substance and fibers.

Resists compression and stretching forces (like in skin and tendons).



1. The Ground Substance:

- The ground substance is mainly water, allows rapid exchange and transport of metabolites.
- It's not only water because according to gravity, this water will pool in the lower part of the body so in fact it's a gel-like structure.
- This water is stabilized by macromolecules (called stabilizing macromolecules), it functions like a jello, it transforms water from liquid state to viscous state. For example, in blood, the ground substance lacks stabilizing macromolecules and lacks fibers, so the extracellular matrix is fluid.
- With different types of connective tissue , we see different components of ECM (like : in blood its ECM is fluid but in bone it's solid because it has high amount of Ca^{++} ions. Blood and Bone are special types of C.T)

- Here we are talking about ORDANIRY C.T (not special types).
C.T which is located directly under epithelium (for example).
- Functions :
 1. Makes the gelatinous material of the connective tissue.
 2. Prevents the movement of microorganism.
 3. Allows the movement of gases and small molecules.

Types of ground substances (macromolecules):

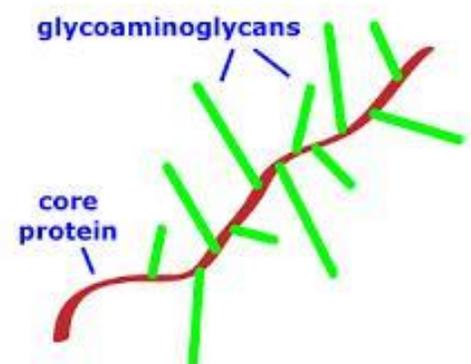
1. Glycosaminoglycans (GAGs).
2. Proteoglycans.
3. Glycoproteins.

1. GAGs:

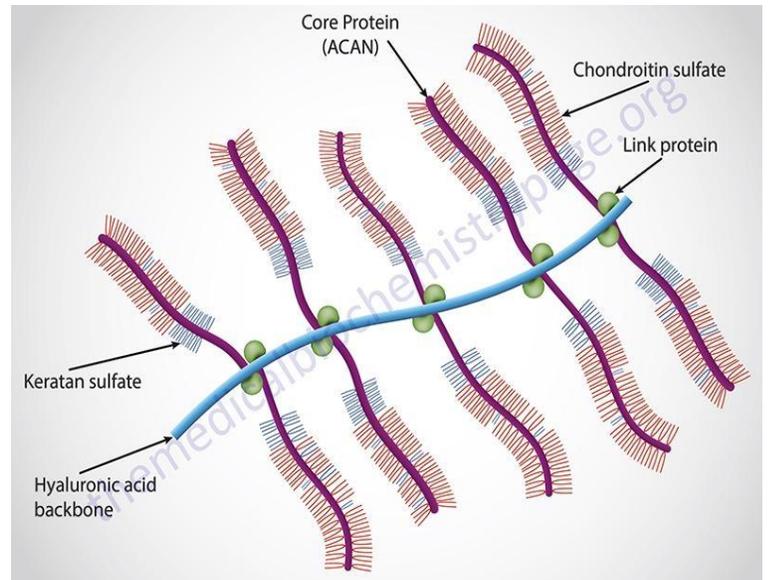
- ✓ Long chains of repeating units of disaccharides (2 sugars), unbranched.
- ✓ Have two types :
 - A. Sulfated: contain sulfate group such as (*keratin sulfate, chondroitin sulfate, dermatan sulfate, and heparin sulfate (heparin)*).
 - B. Non-sulfated: No sulfate group, the only example is: hyaluronic acid, which is a long molecule with very high molecular weight.
- ✓ GAGs are highly negatively charged (polyanions).

2. Proteoglycans:

- ✓ very large macromolecules consists of :
 - proteins (~5%)
 - polysaccharide chains (~95%) belong to *sulfated glycosaminoglycans*.
- ✓ The protein core attaches to many GAGs
- ✓ Functions of Proteoglycans
 - a. Resistance of compression.
 - b. They are stabilizing macromolecules (little amount of it gives water gel state).
 - c. Retardation of movement of microorganisms.
 - d. Act as a filter



- ✓ The high density of negative charges “from GAGs” attracts water, forming a hydrated gel. This gel permits the rapid diffusion of water- soluble molecules but inhibits the movement of large molecules and bacteria.
- ✓ The hyaluronic acid interacts with proteoglycans and makes a backbone for protein cores where the glycosaminoglycans attach, forming a spherical space called a domain.



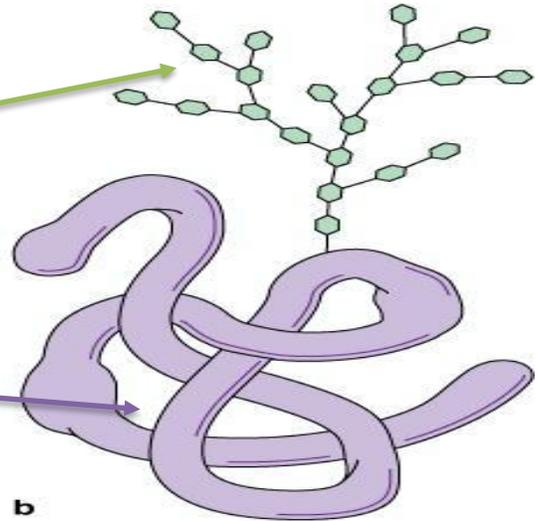
- ✓ Each domain looks like a Bottle Brush: the handle is hyaluronic acid and the bristles are the proteoglycan.
- ✓ Bacteria need to pass between these domains in order to pass through the connective tissue, so that is why we say that the ground substance has **filtering** effect and **prevents the spread of the bacteria**. The pathogenicity of a bacterium is indeed to some extent determined by its ability to find its way through the mesh, and some of the more invasive types produce the enzyme hyaluronidase, this enzyme is able to destruct hyaluronic acid , that destructs this domain and enables bacteria to invade the connective tissue.
- ✓ Hyaluronic acid is used in filling fascia and Scabrities of joints because it attracts water molecules and makes a hydrated gel.

****خشونة المفاصل والحشوات التجميلية.****

3. Glycoproteins:

✓ Consists of : ● polysaccharide chains (~5%) are *branched and short* (oligosaccharides, oligo= few).

● proteins (~95%) .



✓ **Adhesive glycoproteins:**

A. Laminin: connects the integrins of the hemidesmosomes with the other proteins of ECM.

B. Chondronectin: it is found in cartilage. "chondro= غضروف. The connection (adhesion) is between the cells of cartilage and ECM

C. Osteonectin: "osteo means it is in the bone".

D. Fibronectin: it is found in basal lamina, connection between cells and ECM.

□nectin means connection.

2. Fibers of connective tissue:

a) **Collagen fibers**: give strength.

b) **Elastic fibers**: give elasticity

c) **Reticular**: give a network.

"Elasticity means, the ability to restore the original shape after distortion".

- In any connective tissue we can find the 3 types of fibers, but the proportions of these components differ.
- Collagenous connective tissue is composed mainly of **collagen type 1**.
- elastic connective tissue is mainly composed of elastic fibers.
- reticular connective tissue is mainly composed of reticular.

Collagen fibers

■ We have many types of collagen fibers, each type has different distribution than other types.

■ We have **28 types of collagen** “as a protein” , the differ in sequence of amino acids, chains combinations, distribution and modifications.

1. **Collagen type 1** : is the strongest type of collagen, most common. Found in skin, tendons, capsule, and bone. Each **third amino acid** is glycine, also each helix is rich in proline and lysine, hydroxyproline, hydroxylysine, and 1% of the protein is carbohydrates (sugar).
2. **Collagen type 2** : is found in cartilage.
3. **Collagen type 3**: Reticuloendothelial system, in reticular lamina of basement membrane.
4. **Collagen type 4**: in basal lamina.
5. **Collagen type 7**: connects collagen type 3 to 4 in basement membrane.

■ Collagen is a protein, **30%** of the proteins in our body is collagen, the most common protein in our body.

■ Collagen fibers are very strong and nonextensible, to tear them you need hundreds of kilograms per 1 cm², leather for example consists of Collagenous Fibers - fibers that originate from the skin of large mammals.

■ All Collagen fibers are glycoproteins.

■ Collagen fibers are colorless, the bulk accumulation of them appears white (the tendon is composed of collagen type 1, grossly it appears whitish).

■ ***Birefringence, that gives the white colour for the bulk.***

■ With H & E, it appears **acidophilic** (undulating course of longitudinally striated pink bundles, **eosinophilia**).

■ In the section of connective tissue, **eosinophilia**, is the **collagen fibers** because:

**Cells have basophilic nuclei (purple).

1) the **lightly stained** nuclei are **fibroblasts** "active cells".

2) the **darkly stained** nuclei are **fibrocytes** “inactive cells”.

**Other components appear like unstained.

How does the collagen is synthesized?

- 1- **In Fibroblast**, DNA is transcribed for collagen mRNA in nucleus.
- 2- mRNA is translated in the rough endoplasmic reticulum.
- 3- **Glycosylation** : the addition of glucose to the protein and **Hydroxylation** : the addition of a hydroxyl group occur in the ***Rough Endoplasmic Reticulum.***
- 4- It gets modified in the **Golgi apparatus** (addition of sugar

glycosylation).

5- It gets exported from the cell by **exocytosis**, the product is called **Procollagen** (*not the final form of collagen, pro means before*).

- Procollagen is made up of :
 - a) triple alpha helical chains.
 - b) nonhelical parts on the edges.

6- A certain enzyme in the ECM cuts these edges, after cutting the edges it's called **Tropocollagen**. * helical chains with regular gaps between each two tropocollagen.

7- The Tropocollagens align next to each other and above each other to form **Collagen Fibril** "smaller than the fiber". **Cross Striations** appears in collagen fibril under electron microscope, these striations are formed by the regular gaps between tropocollagens molecules because of the *accumulation of the stain in these gaps, that forms dark regions*.

8- Collagen **Fibrils** aggregate to form **Collagen Fiber**.

9- Collagen **Fibers** aggregate to form **Collagen Bundle**.

NOTE : The process of synthesizing collagen is a very long process and has many steps; any defect in any step results in defective collagen (disease).

For example, **Hydroxylation step needs Vitamin C**, the source of this vitamin is the diet, our body doesn't synthesize it.

Only **collagen type 1** can form collagen bundles.

Collagen type 3 forms fibers (thin fibers), Collagen type 2 forms fibrils,

Collagen type 4 in basal lamina forms sheets.

* Features of Tropocollagen :

1- 3 helical chains, edges are cut. Each molecule has head and tail, so the arrangement is (head to tail, head to tail and so on), with a regular gap between two tropocollagens.

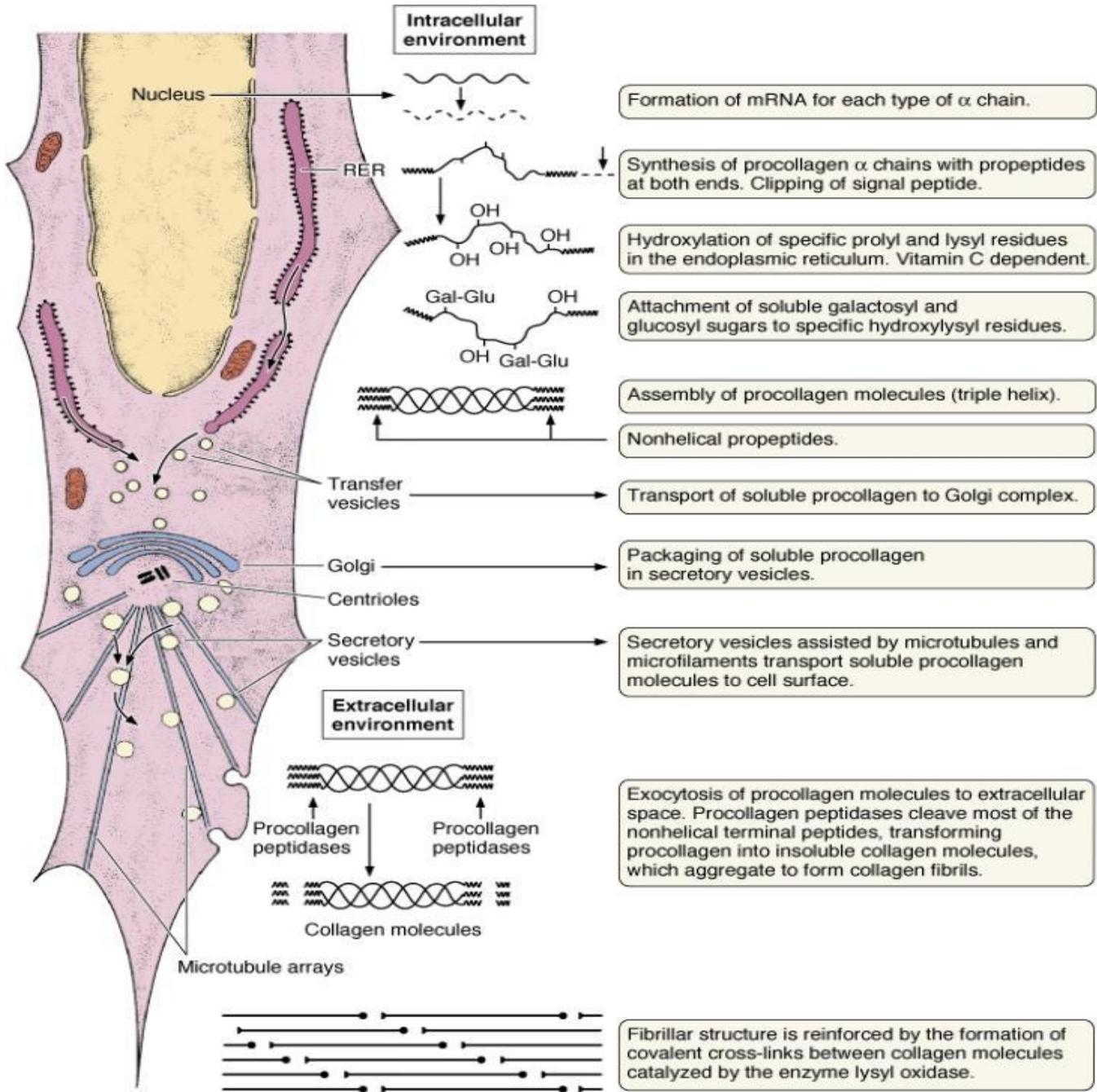
2- The longitudinal areas of completely overlapping tropocollagens are called **overlapping areas** (appear light in colour).

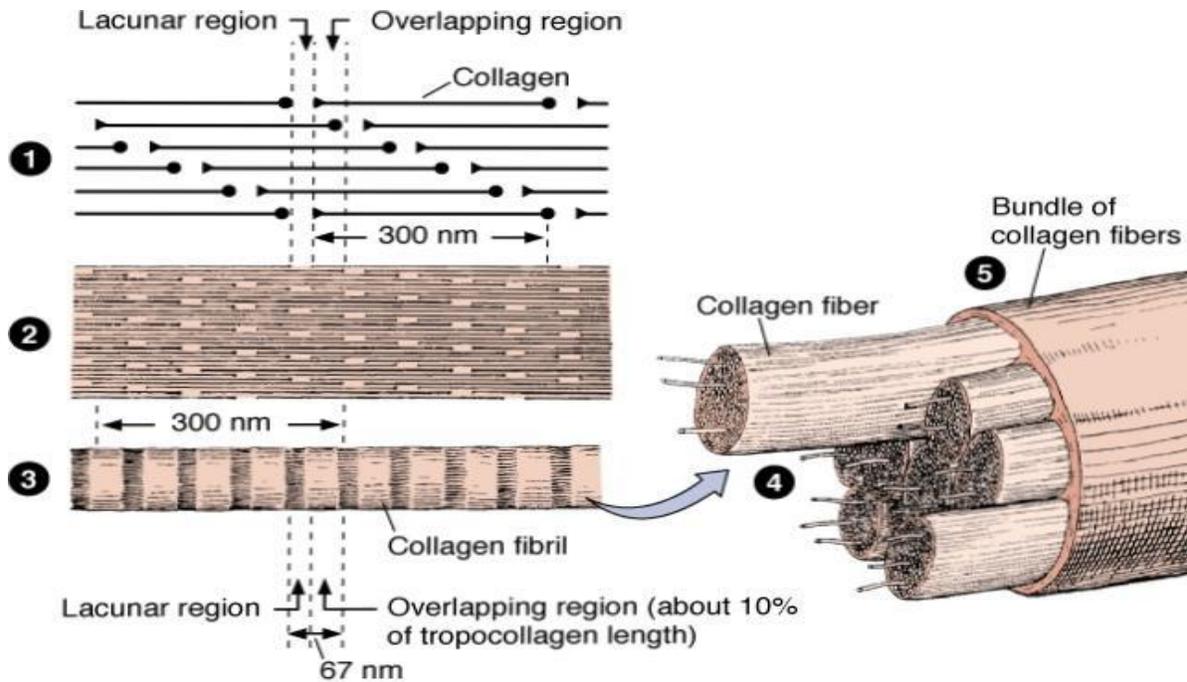
3- The longitudinal areas where there are gaps between tropocollagens are called **non-overlapping areas** (appear dark

in colour).

4- the non-overlapping areas appear darker and overlapping areas appear lighter (striations).

Using E.M: cross banding at 67 nm = overlapping + non-overlapping.





Collagen bundle > Fibers > Fibrils > Tropocollagen > 3 Helical polypeptide chains, α -chains.

Clinical applications:

- ✓ At the surgical incision site, the site is filled with collagen fibers synthesized by fibroblasts. This is called scar formation, and it's part of the *normal healing process (formation of pink line then it becomes white due to the orientation of collagen)*. The fibrocytes in the connective tissue are activated due to injury, so become fibroblasts.

Keloid: The excessive formation of collagen during healing process.

- I. The fibroblasts are **genetically modified** to form and secrete high amount of collagen I.
- II. The incision site appears larger in size, pinkish in colour, elevated from the surface and with ugly appearance.
- III. Keloid is common in Africans and can't be removed surgically.

- ✓ ***Scurvy*** مرض الإسقربوط : deficiency in vitamin C, which is very important in the hydroxylation step. This result in defective collagen,
 - I. The patient has hemorrhage (bleeding) in a lot of areas.
 - II. The patient is anemic and his gums are bleeding.

- ✓ ***Ehlers–Danlos syndrome*** : caused by a defective collagen; the joints become **hyperflexible and hypermobile**.

Elastic fibers

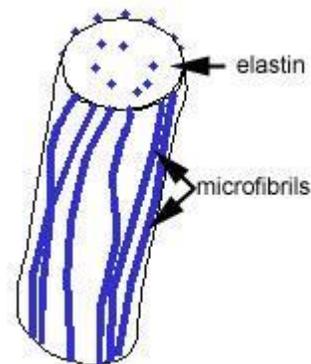
- ❖ The main function of elastic fibers is to provide elasticity, are able to restore the original shape of the tissue after distortion.
- ❖ As a rubber band, stretched when a force is applied and relaxed (back to its original shape) when the force is released, they are **extensible**.
- ❖ Elastic tissue can deteriorate (be damaged) by three factors:

1-The elasticity of tissue decreases with age, **e.g.** : This effect is easily demonstrated by recruiting two volunteers, one youthful and one elderly. Pinch up a bit of skin on the back of each person's hand and then watch how quickly the skin returns to its original position when released.

2-Sun exposure: the elastic fibers (like a rubber band) loses their resilience and elasticity when exposed to sunlight for a long period of times.

3-Being digested by pancreatic enzyme elastase.

- ❖ Elastic fibers consist of individual microfibrils “blue in the picture”, which are embedded in an amorphous matrix “colorless in the picture” .
- ❖ Under EM elastin protein appears as *electron-dense amorphous* material (with no shape).
- ❖ H&E stain doesn't view elastic fibers *unless* they are present in *high concentration*. “we use special stains like : Orcein and resorcin Fuchsin. (elastin stain) and appears brown-black”.
- ❖ Appears yellow in fresh tissue (as large amount is present, *flavum: yellow*)
- ❖ Components of elastic fiber (2 proteins):
 1. 90% of the fibers are elastin gives Elasticity.
 2. 10% of the fibers mostly is microfibrils of fibrillin protein gives Stability (resistant to



boiling).

** Remember that elastic fibers as well as most of ECM compounds are produced by the fibroblasts (with some exceptions according to the site).

❖ Elastic fibers are found in all types of C.T. In some tissues they are the predominant fibers and the tissue is called elastic tissue examples:

1-Types of ligaments contain elastic fibers as the predominant fibers like the ligamentum flavum (connects the vertebrae of the vertebral column).

2-Types of cartilages are composed mainly of elastic fibers and called Elastic Cartilages, example Ear Pinna, and ***Epiglottis*** at the back of the tongue that closes the respiratory tract so that food can enter the esophagus. *EPIGLOTTIS*: لسان المزمار

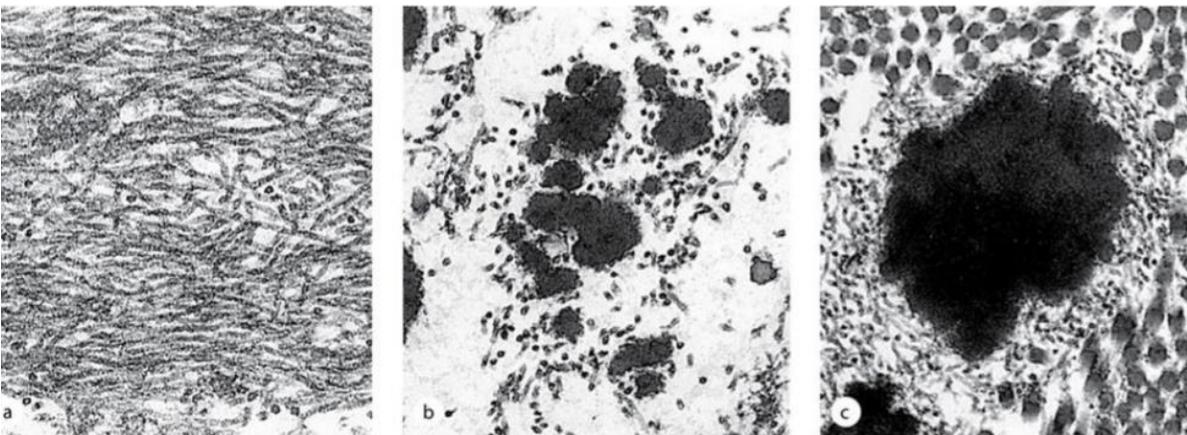
3-In the wall of large arteries like *aorta* to ensure the recoiling of blood vessels after dilation.

4-The Skin.

How does the elastic fibers is synthesized ?

1. fibroblasts form the microfibrils.
2. elastin protein (characterized by being a coiled protein) deposits and accumulates over these microfibrils.

In the picture : a is microfibrils , b is elastin proteins, c is an elastic fiber.

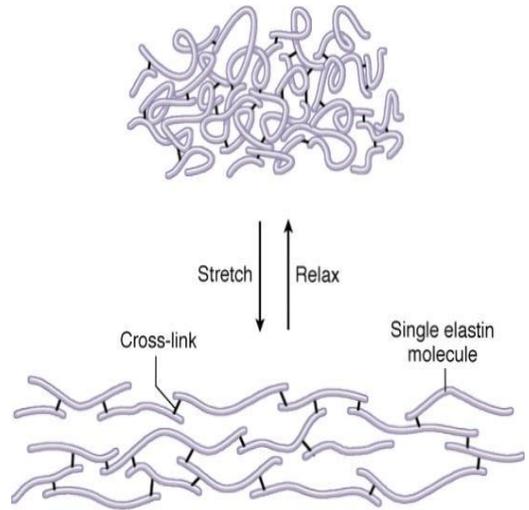


Note: a ligament connects bone to bone and is usually (not always)

made of dense collagenous connective tissue.

How does elastic fibers perform the elasticity function?

- 1) Elastin proteins are cross-linked by a special type of link called desmosine.
- 2) The Elastic fiber is stretched when a certain force is applied.
- 3) The Elastic fiber restores to its original state when the force is released.
- 4) There is always a certain minimal amount of collagen fibers, even in elastic tissues, which **prevents overstretching** or provides what is called a **mechanical stop**, because these fibers are strong and nonextensible.



Reticular Fibers

- Reticular fibers were initially thought to be completely different from collagen fibers, Because of their different staining characteristics.
- Cross- striations with the same periodicity as in collagen fibers type 1 are visible using EM, but a different type of collagen BECAUSE :

- They give **PAS positive reaction**, so have high sugar content.

All the rest of collagen fibers types contain carbohydrates but in minimal amount (about 1% only), thus, collagen fibers are considered glycoproteins, but collagen fibers type three (reticular fibers) contains much higher amount of carbohydrates (10% carbohydrates).

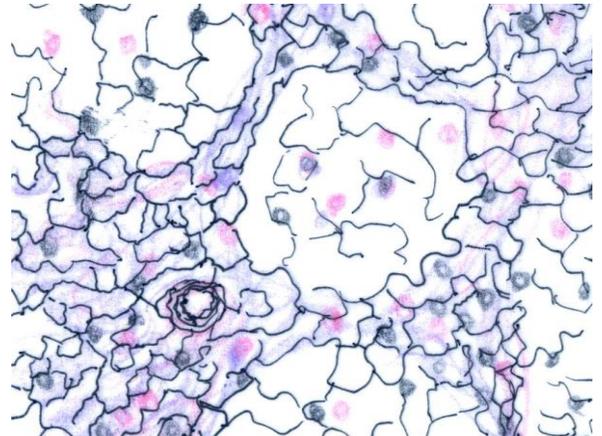
- They Consist mainly of type III collagen, forming fine networks (*thin, short and branching*) instead of thick bundles.

- They can be stained with Silver Nitrate, so they are *Argyrophilic*.

Silver Impregnation is a process of staining includes adding silver to the tissue, because these fibers have a high amount of carbohydrates, and these carbohydrates are negatively charged, thus, they are going to interact with the silver and cause its reduction, which results in deposition of silver

inside the tissue, so that the reticular fibers can be seen as **black threads**.

- Reticular fibers are made by *Reticular Cells* : a special name for *fibroblasts* which are modified to secrete Reticular fibers, are found mainly in Reticular tissue.
- The cells that are specialized in secreting reticular fibers are called reticular cells, which are modified fibroblasts.
- Reticular fibers are usually in the form of **fibrils** . Also, they can aggregate to form **thin fibers**. Reticular fibers are extensible.



- They are found in :
 - 1) Reticular lamina of basement membrane.
 - 2) RES organs (*Reticulo- endothelium system* : consists of blood channels lined by Endothelium and supported by Reticular tissue rich in reticular fibers. These organs are bone marrow, liver, lymph nodes and spleen, have a meshwork of reticular fibers which acts as the framework for these organs (supportive stroma and supporting individual cells) .

- The function of reticular fibers :
 1. Forming the stroma of certain organs (like lymph nodes and spleen).
 2. Supporting individual cells.
 3. Allowing the movement of cells and molecules, Ex: lymphoid tissue, like in lymph nodes, is filled with cells which are called lymphocytes. So, these fibers form spaces in which these cells "lymphocytes " can move freely within the tissue).

Comparing between the three types of fibers:

Collagen fibers : type 1, give strength, appear as pinkish bundles by staining with H&E and their bulk is grossly white.

Elastic fibers: give elasticity, need special types of stains like Orcein and resorcin (elastin stain) and they are grossly yellow.

Reticular fibers : collagen type 3, form a network, delicate network (meshwork) of fibers, provide the structure of certain organs. stained + with PAS, Silver stain.

Different proportions of these components in the connective tissue we give different types of C.T : Reticular, Elastic, and Collagenous (Dense) connective tissues.

The classification of the connective tissue, the standard classification scheme is **based on composition**.

1- Connective Tissue Proper

- Loose Connective Tissue.
- Dense Connective Tissue :

- 1) Dense Irregular Connective Tissue.
- 2) Dense Regular Connective Tissue.

2- Special Connective Tissue (has similar characteristics of the connective tissue) :

- **Cartilage** ▪ **Reticular tissue** ▪ **Elastic tissue** ▪ **Adipose tissue**
- **Blood** (cells and extracellular matrix with no fibers and stabilizing macromolecules like GAGs, that's why it's fluid).
- **Bone** (cells and mineralized extracellular matrix, that's why it's hard).

We refer to a special type of connective tissue by its name.

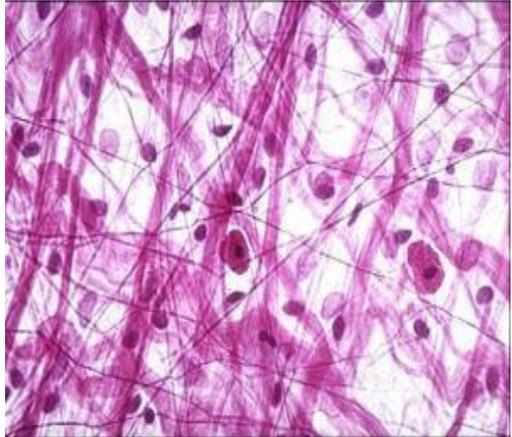
3- Embryonic connective tissue:

- Mesenchymal connective tissue • Mucous connective tissue.

Connective tissue proper

- i.* It contains the components that were mentioned (fibers, cells, ground substance), with different proportions.
- ii.* When we say connective tissue we mean connective tissue proper.
- iii.* If the main component of tissue is collagen fibers then it is called Dense Collagenous Connective Tissue.
- iv.* If the main component is cells or ground substance (or equal proportions) then it is called Loose Connective Tissue.

A. Loose connective tissue

- Called also areolar connective tissue.
 - Typically contains cells, fibers and ground substance in equal amounts (thin or delicate fibers).
 - Supports epithelium (lamina propria of mucus membrane below epithelium).
 - Surrounds small blood vessels.
 - Fills spaces between muscle and nerve cells.
 - It's flexible because it has high amount of ground substance, high amount of water. The extracellular matrix is gel like, not very strong.
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- Not very resistant to stress because it has low amount of collagen.
 - It has more regenerative power than dense connective tissue because it has more cells and more vascularized.

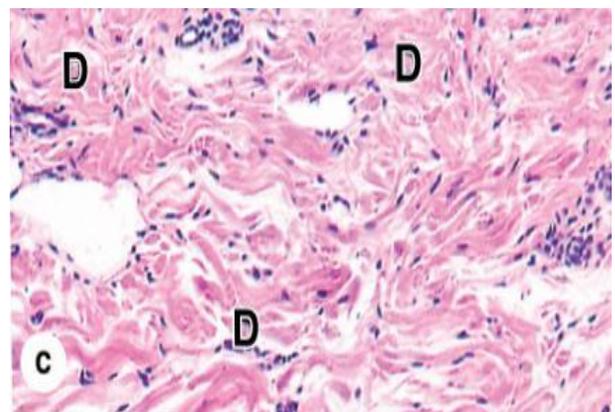
• Function:

1. **Diffusion** (medium for transport) because it has a lot of spaces filled with ground substance, Found around blood vessels, directly under epithelium layer, highly vascular (high amount of capillaries) in order for the molecules to pass from the blood vessels to the epithelium and backward.
2. **Second line of Defense** below basement membrane of epithelial cells because it has inflammatory cells (e.g. macrophages)
3. **Filling spaces** inside any organ.

**Lamina propria is a special name for the loose connective tissue of mucus membranes "mucosa".*

B. Dense Irregular Connective Tissue (collagenous)

- Mostly composed of collagen fibers,
- Bundles of collagen fibers are randomly interwoven with no definite orientation
- Collagen fibers add strength to the connective tissue so it's very resistant to compressional forces and tensile forces.
- Provides powerful resistance in

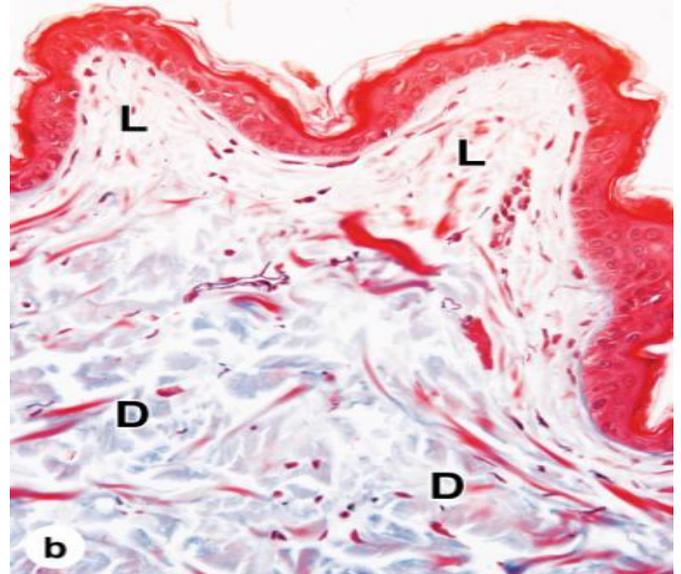


many directions (according to the orientation of the fibers).

○ Locations :

- a. Deeper layer of dermis of skin: below the loose connective tissue layer (exposed to many forces in different directions).

First layer (superficial) of connective tissue is loose, the next layer (deeper) is dense irregular connective tissue (to give strength for loose and epithelium)
Epithelium of the skin is epidermis, and the connective tissue of the skin is dermis.



- b. Organ capsules : capsules that surround any organ are whitish because they have high amount of collagen but the orientation of the collagen fibers is irregular.

- c. Submucosa (mucosa=mucous membrane= epithelium and thin layer of loose connective tissue (lamina propria))

C. Dense Regular Connective Tissue (collagenous)

- i. Collagen Fibers arranged in parallel bundles (oriented in certain direction)
- ii. Provides resistance to prolonged or repeated stresses exerted in the same direction
- iii. Fewer cells and ground substance and most of the cells found are fibroblasts not inflammatory cells.
- iv. Locations :



Ligaments: connect bone to bone, usually are collagenous (whitish in color) dense regular type of connective tissue except certain types of ligaments (elastic/yellowish in color).

Tendons: connect muscle to bone.

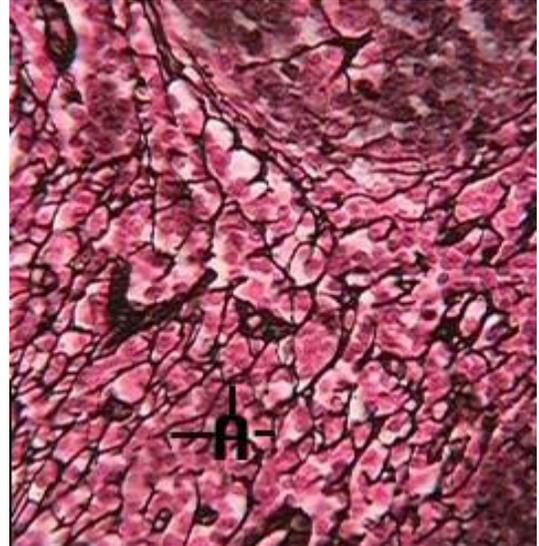
Aponeurosis: flat tendon (sheet like) instead of cord like structure.

» » Regeneration Power is variable according to the type; **loose** has a **higher regeneration power** than dense connective tissue because it has

higher amount of cells and capillaries in comparison with **dense** connective tissue (fibers and little amount of cells). If a tendon gets injured usually the regeneration is poor because it's less vascularized, and repair of damaged tendons is very slow.

Reticular tissue

- consists of reticular cells (modified fibroblasts) and a network of reticular fibers formed by them
- forms the structural framework in which the cells of the organ are suspended
- in the liver, bone marrow, lymph nodes and the spleen



Embryonic connective tissue

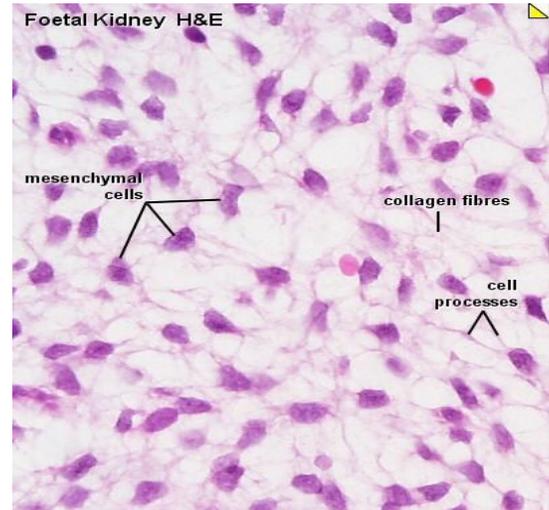
The connective tissue that presents in embryo.

1. Contains star or spindle shaped mesenchymal cell (stem cells),
2. gel-like ground substance with fine fibers (more ground substance).
3. the Embryo has three germinal layers : *Ectoderm*, *Mesoderm*, *Endoderm*.

a) The mesenchyme connective tissue

- ✓ **The Mesoderm** differentiates into a tissue called *Mesenchyme* or Embryonic Connective Tissue.
- ✓ Mesenchyme forms the filling material of the early embryo and it is *the common origin* of **all** other connective tissue types.
- ✓ With fetal development, Mesenchyme forms the connective tissue between and within the developing tissues and organs.

- ✓ The ECM of mesenchymal C.T contains more ground substance than fibers, it's a gel-like structure because it has large amount of hyaluronic acid, chondroitin sulfate (sulfated GAG) and water.
- ✓ Mesenchymal stem cells are characterized by having a little amount of cytoplasm with fine processes and large oval or rounded nuclei.
- ✓ Mesenchymal Cells interconnect by slender cell processes.
- ✓ Mesenchymal cells have stem cell properties, i.e. they are able give rise to other cell and tissues types.
- ✓ Mesenchyme Tissue **doesn't contain fibroblasts.**
- ✓ Mesenchymal cells can differentiate into many types of connective tissue cells (pluripotent), like :



1. Osteoblasts which form the bone.
2. Fibroblasts which form the proper connective tissue.
3. Adipoblasts which form the adipose tissue.

***Remember that epithelium is derived from the three germinal layers which is a characteristic for it, while connective tissue is derived from mesenchyme (or mesoderm).

- ✓ Mesenchymal cells of this tissue can form bone, blood and different types of C.T.

Mesenchymal tissue or embryonic tissue is found only in embryo while after birth we can't find mesenchymal tissue because *all mesenchymal tissues have differentiated into different types of connective tissue.*

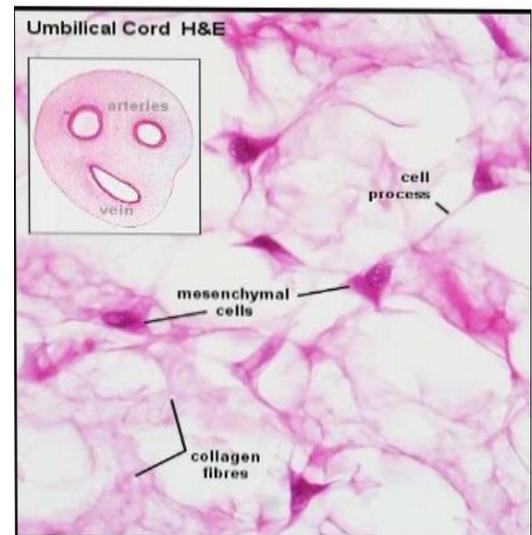
EXCEPT in two places : □□ The Pulp of the Tooth in adults (as a **tissue not individual stem cells**). □□ The Umbilical Cord.

***the umbilical cord connects the placenta of the mother with the umbilicus of the fetus, has two arteries and one vein. الحبل السري

- ✓ Mesenchymal Cells have large nuclei and fine processes.

b) The mucous connective tissue

- ✓ **Muroid Connective Tissue** is the mesenchymal C.T of the **umbilical cord** because ITS GROUND SUBSTANCE IS MUCOUS-LIKE, made up of *mucopolysaccharides* (hyaluronic acid and chondroitin sulfate).
- ✓ **Muroid Connective Tissue** is also called Wharton's jelly or mucous connective tissue.
- ✓ **Muroid Connective Tissue** forms a compliant cushion around the vessels of the umbilical cord.
- ✓ Umbilical Cord Banking simply involves *collecting blood left in newborn's umbilical cord and storing it for future medical use* (cord blood and/or tissue) (regenerative medicine).



***Stem cells mean undifferentiated cells that can become a **variety of cells.**

Types of GAGs

Read only

GAG	Distribution
Hyaluronic acid	Most connective tissue, cartilage, dermis, synovial fluid.
Keratan sulfate	Cartilage, cornea, intervertebral disc.
Heparan sulfate	Blood vessels, lung, basal lamina
Chondroitin 4-sulfate	Cartilage, bone, blood vessels
Chondroitin 6-sulfate	Cartilage, blood vessels, umbilical cord.
Dermatan sulfate	Skin, heart valves, blood vessels
Heparan sulfate (Heparin)	Mast cell granules, basophils, liver lung, skin.

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