بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

HISTOLOGY

وَلَقَدْ خَلَقْنَا الْإِنسَانَ وَنَعْلَمُ مَا تُوَسْوِسُ بِهِ نَفْسُهُ ۖ وَنَحْنُ أَقْرَبُ إِلَيْهِ مِنْ حَبْلِ الْوَرِيدِ

MID | Lecture 10a

Fibers and Ground substance

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Color coding used in the modified:

- *Black: the original slides*
- *Maroon: the doctor’s explanation/words*
- *Gray: additional information and explanation*
- *Red: important information*

Connective Tissue Fibers

1. Collagen:

- Form various extracellular fibers, sheets, and networks.
- Extremely strong and resistant to normal shearing and tearing forces.
- Collagen is a key element of all connective tissues, as well as epithelial basement membranes and the external laminae of muscle and nerve cells.
- Most abundant protein in the human body, representing 30% of its dry weight.
- A family of 28 collagens exists in vertebrates.
- **Collagen provides strength and elasticity and it resists tensile and shearing forces, making it suitable for ligaments that connects bones.**

Collagen types

There are 3 types of collagen in our bodies :

I. **Fibrillar collagens**, notably collagen types I, II, and III. Form structures such as tendons, organ capsules, and dermis.
The fibrillar collagens are extremely strong.

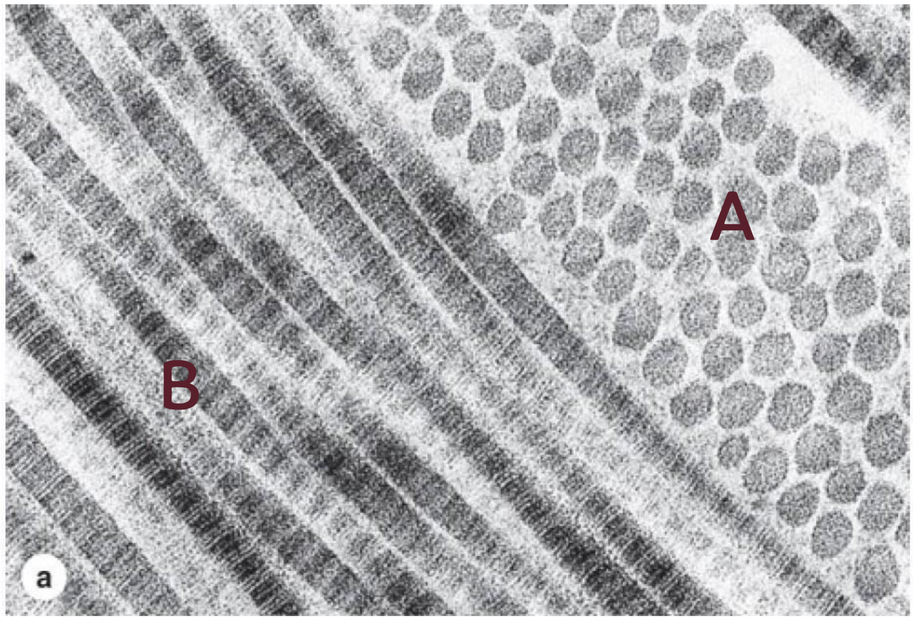
II. **Network or sheet-forming collagens** such as type IV collagen have subunits produced by epithelial cells and are major structural of external laminae and all epithelial basal laminae.

III. **Linking/anchoring** collagens are short, such as VII, IX collagen , they help linking proteins and different materials together, one example is collagen VII which links proteins and elements in the reticular lamina.

- **Collagen represents about 30% of the body's dry weight and is considered very strong.**
- **There are 28 types of collagen, divided into three families, each with different characteristics.**
- **Collagen provides strength and elasticity, making it suitable for the ligaments that connect bones.** (Ligaments contain mainly collagen 1)

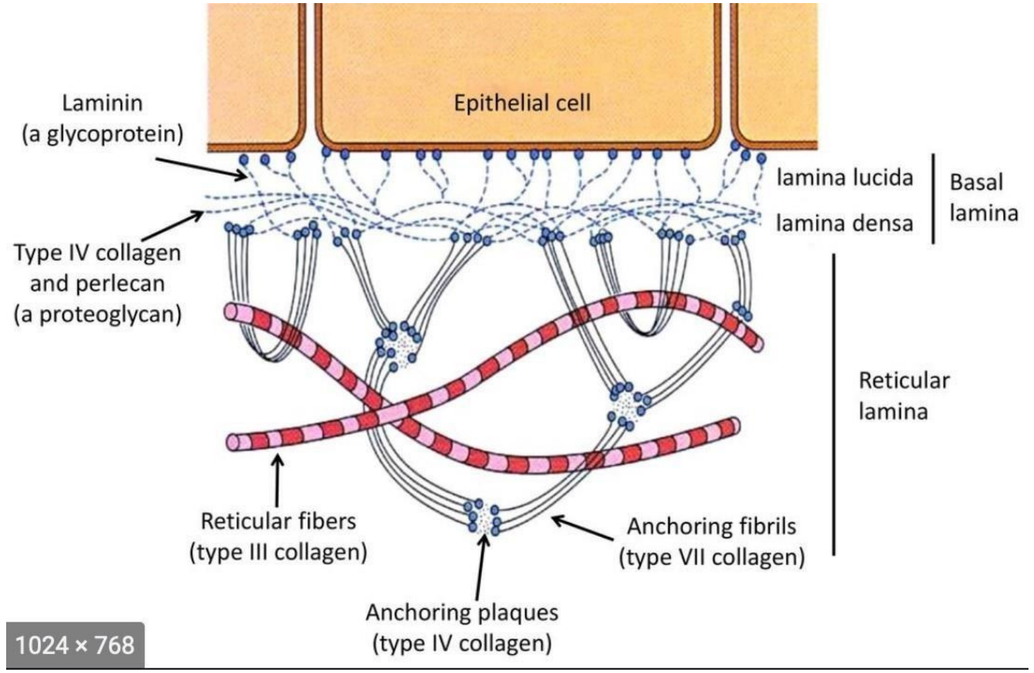
Collagen types

In the longitudinal sections , we can see dark and light regions, that's called striation of collagen.



Taken from TEM. This shows cross-section collagen fibers (A) and longitudinal too (B).

You can see collagen IV present in basal lamina, and type VII where it helps binding (linking) proteins and elements in the reticular lamina.



Collagen Assembly

1. **Procollagen subunits,** Rodlike triple-helix collagen molecules, each 300-nm long. They self-assemble in a highly organized, lengthwise arrangement of overlapping regions **to make structures called collagen fibrils.**

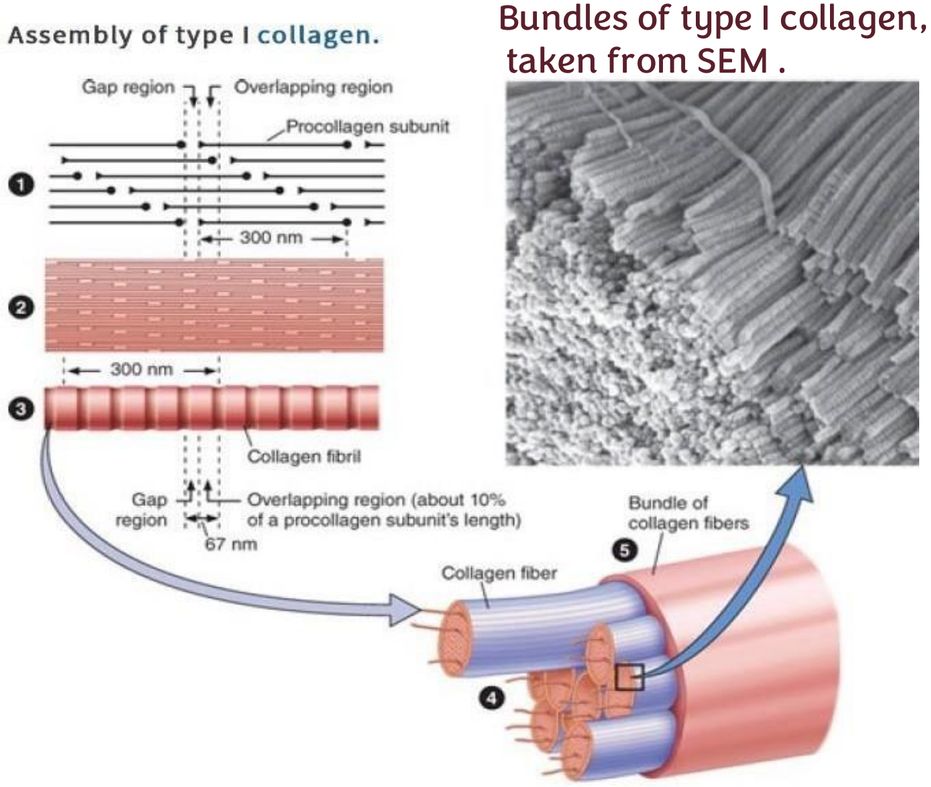
2. The regular, overlapping arrangement of subunits continues as large collagen fibrils are assembled.

3. This structure causes fibrils to have characteristic cross striations with alternating dark and light bands when observed in the EM.

4. Fibrils assemble further and are linked together in larger collagen fibers visible by light microscopy.

5. Type I fibers often form into still larger aggregates bundled and linked together by other collagens.

The concept of overlapping in collagen means that procollagen molecules do not align end-to-end in a perfectly straight line. Instead, they are arranged in a staggered, partially overlapping pattern, where each molecule is slightly shifted relative to the next. This creates regions with greater overlap, which are more dense and appear darker, and regions with less overlap, which are less dense and appear lighter under the electron microscope. This organized arrangement forms collagen fibrils, which later assemble into larger fibers.



Bundles of type I collagen, taken from SEM .

- The pro collagen subunits arrange in a way that forms small gaps between each subunit, and this gap is not being overlapped on the second row or the ones below of the procollagen subunits, it takes many rows of those procollagen lines for 2 gaps to meet, and when this overlapping of the gaps happens, it will be expressed as a light region in the electron microscope . (it cant be visualized in light microscopes).

Largest Smallest

- Collagen bundles –> collagen fibers–> collagen fibrils –> procollagen subunits.

- Collagen fibers are composed of procollagen units and gather in larger collagen structures.
- Network fibers are a type of collagen found in thin organs, such as the endocrine and pancreatic glands.
- They are difficult to visualize with ordinary stains and require special silver salts to be seen under a microscope.

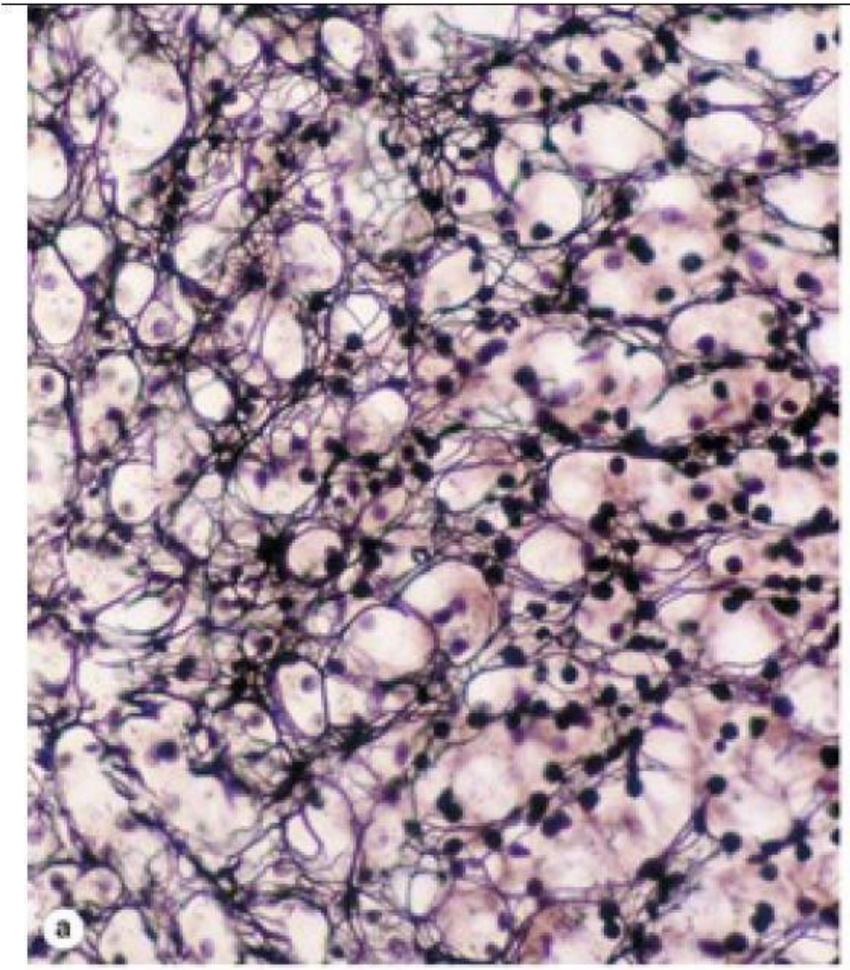
Connective Tissue Fibers
2. Reticular

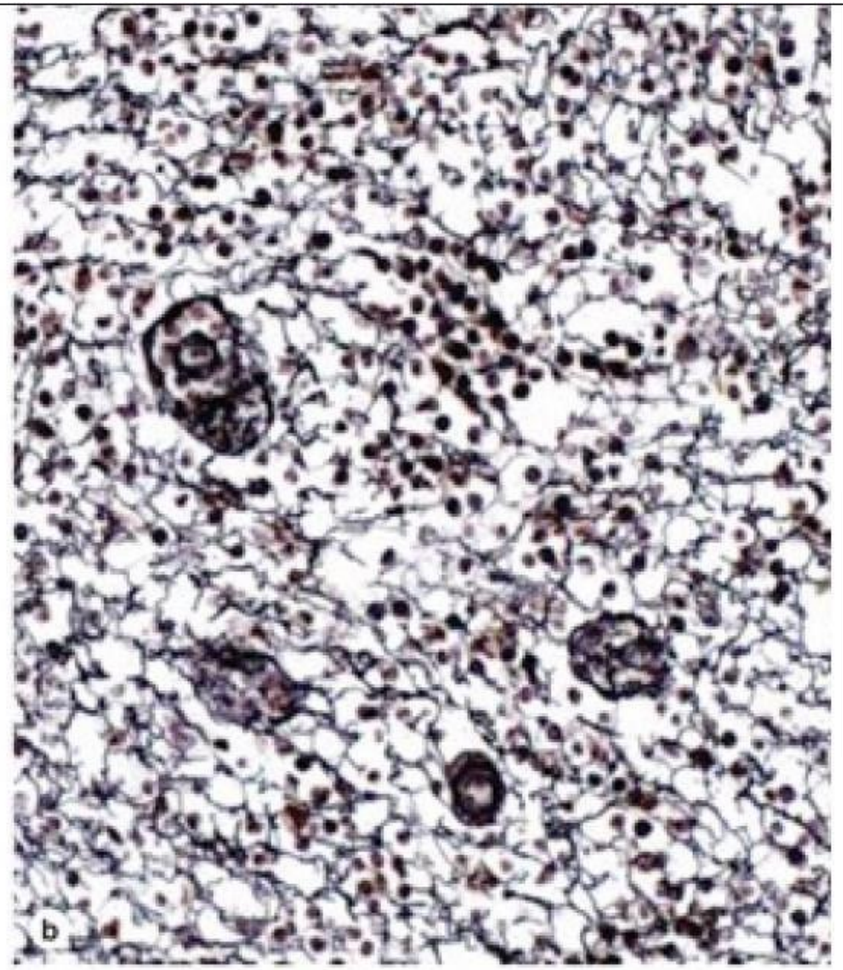
Reticular fibers are a type of connective tissue fiber that are thin and provide a supportive framework for various organs, particularly in lymphoid tissues like the spleen and lymph nodes. Silver staining makes these reticular fibers appear black or dark brown against a light background.

- Found in delicate connective tissue of many organs, notably in the immune system.
- Consist mainly of collagen type III, which forms an extensive network.
- Seldom visible in hematoxylin and eosin (H&E) but are stained black after impregnation with **silver** salts. Collagen I and II could be easily stained with H&E or trichrome staining ,unlike reticular fibers
- Periodic Acid-Schiff (PAS) positive-----due to the high content of sugar chains in reticular fibers.
- Reticular fibers contain up to 10% carbohydrate as opposed to 1% in most other collagen fibers.
- Produced by fibroblasts.
- Surround adipocytes, smooth muscle and nerve fibers, and small blood vessels.
- Serve as the supportive stroma for the parenchymal secretory cells, liver and endocrine glands. One of the best locations to study the reticular fibers is in the liver, where it creates 3d structures where they can protect and house the liver cells, which are called the hypatocytes.
- Stroma of hemopoietic tissue (bone marrow), the spleen, and lymph nodes

Reticular fibers

Lymph nodes- silver stain, one of the rich structures with reticular fiber.





The dark black spots are the nuclei.
Those fine black-ish irregular structures are the reticular fibers, these create tiny compartments where they can support the interior delicate cells

The image on the right is low magnified image, whereas the one on the left is high magnified

Connective Tissue Fibers

3. Elastic

Elastic fibers are Known for their stretchability : (the ability to stretch the fiber to a certain limit and they can recoil back).

- Thinner than the type I collagen fibers and form sparse networks interspersed with collagen bundles in many organs (subject to regular stretching or bending).
- Have rubberlike properties that allow tissue containing to be stretched or distended (lungs).
- In the wall of large blood vessels, especially arteries, elastin also occurs as fenestrated sheets called **elastic lamellae**.
- Elastic fibers and lamellae are not strongly acidophilic and stain poorly with H&E unless you have a good amount of them, but if you have a scant amount of them, they will stain poorly.

- Stained more darkly than collagen with other stains such as orcein and aldehyde fuchsin. Orcein will appear as a darker (brownish or blackish) color , while aldehyde fuchsin will appear maroon or red color.

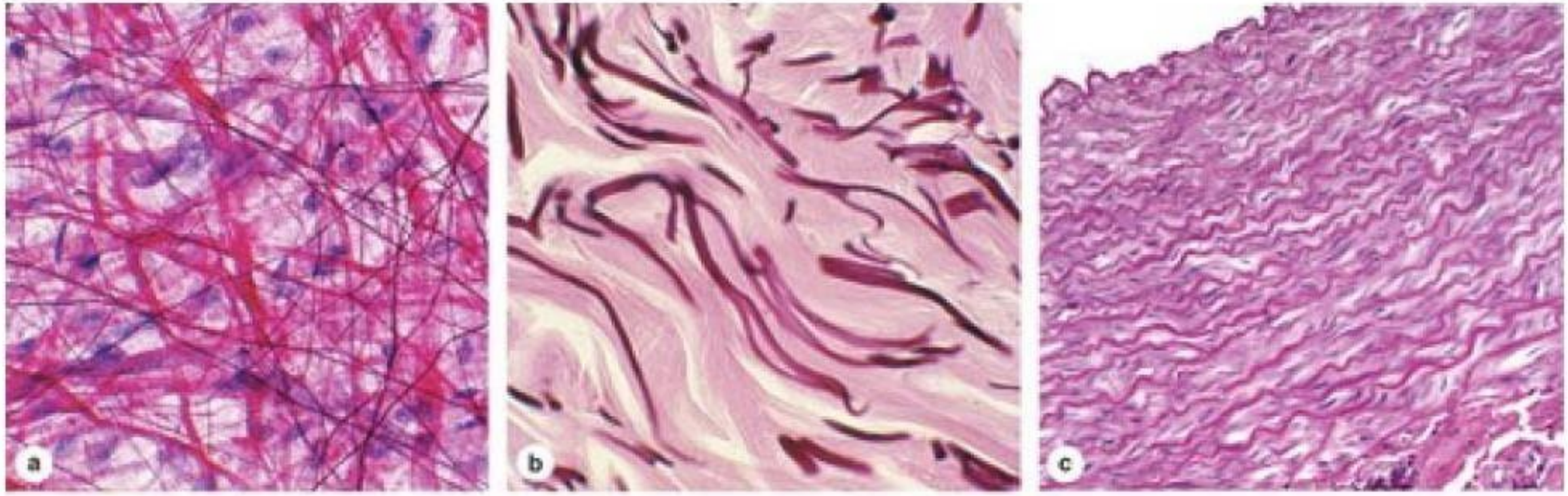
- Elastic fibers are found in the body where stretching is needed, such as:

I. in the lungs (when we inhale the lungs slightly increases in size , this stretching is accomplished because of the elastic fibers in the lungs).

II. Large blood vessels such as the aorta (they rely heavily on elastic fibers because elastic fibers enables them to stretch slightly so they can accommodate the incoming blood from the heart)

III. The dermis (the dermis is the connective tissue that support under the epidermis, that second layer of skin). The skin is subjected to a lot of stretching and pulling forces, so the presence of elastic fibers this important to allow stretching of the skin. IMPORTANT: the dermis is rich with collagen fibers and elastic fibers.

Connective Tissue Fibers/Elastic

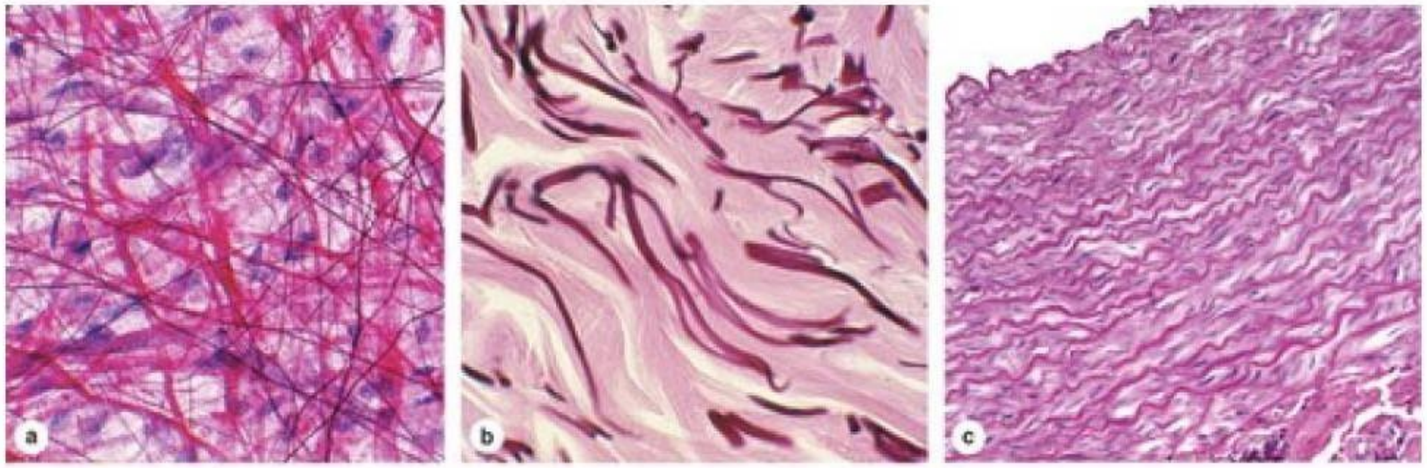


A- Hematoxylin and orcein)
B-Aldehyde fuchsin)
C- H&E

I. Image A : This is taken from the mesentry, you can identify the cells by hematoxylin staining (you see the nuclei), the collagen fibers are the thick ones while the delicate and thin ones are the elastic fibers. Elastic fibers appear in a dark color here because they are stained with orcein.

II. Image B : elastic fibers appear in dark purple or black color. while collagen fibers appear as pale pink, filling the space between the dark and thick elastic fibers .

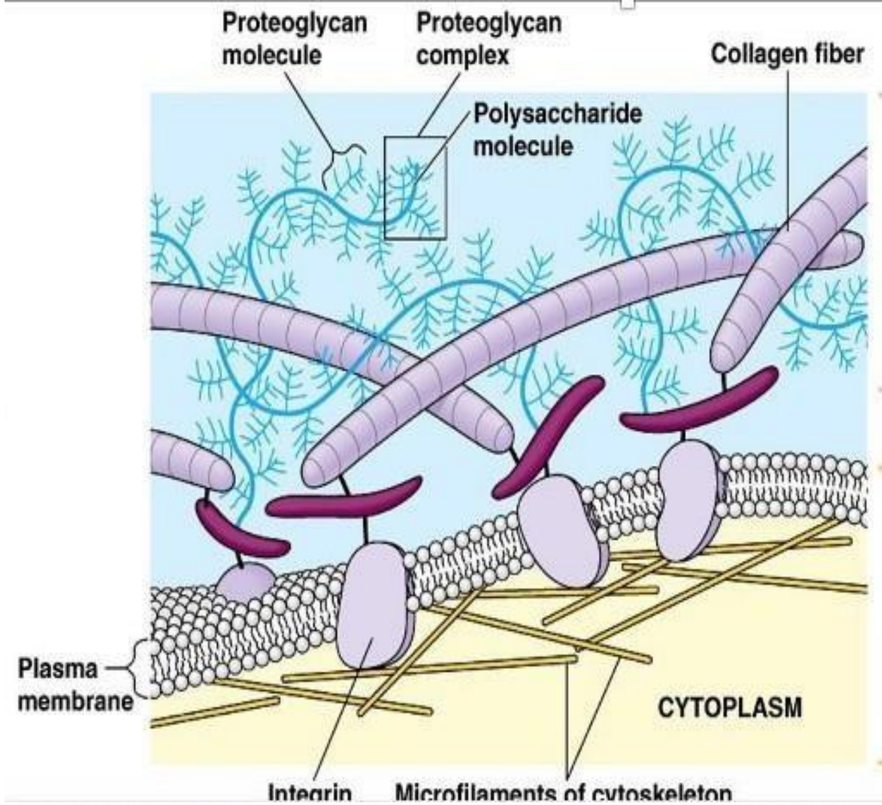
III. Image C : This is a section from the aorta (part of the wall of the aorta) , you can see the lumen , and those wavy dark pinkish lines are elastic lamellae. We have a good amount of elastic fibers stacked and compact in the elastic lamellae, that's why it is not poorly stained with H&E and it tends to show more color, unlike elastic fibers stained with H&E running in between smooth muscles and fibroblasts.



A- Hematoxylin and orcein
B-Aldehyde fuchsin
C- H&E

Ground Substance

- A semi- fluid gel (highly hydrated) and transparent material
- The ground substance of the ECM is a highly hydrated (with much bound water), transparent, complex mixture of three major kinds of macromolecules: glycosaminoglycans (GAGs), proteoglycans, and multiadhesive glycoproteins. Laminin and fibronectin
- Filling the space between cells and fibers in connective tissue.
- Allows diffusion of small molecules.
- Because it is viscous---lubricant and a barrier to the penetration of invaders.



Electron microscope images show how network fibers are assembled from smaller subunits.

- We don’t have empty space in our bodies , that’s why ground substance exists between fibers and cells in connective tissue .

- It’s a semi-fluid gel because it’s highly hydrated, which comes from the abundance of sulfate and lots of negative charges carried on molecules in some types of ground substance. This is extremely important because:

A. it offers lubrication and that is seen in the umbilical cord in the embryo, so that it will offer protection to the important blood vessels in the umbilical cord.

B. Being viscous or gel-like harbors the advancements and movements of microorganisms so it halts their abilities to do more destruction.

- The presence of ground substance allows diffusion (from the connective tissue to the overlying epithelium) of different molecules such as glucose or amino acids or whatever the epithelial cells need.

GAGs

- GAGs (mucopolysaccharides) are long polymers of repeating disaccharide units, usually a hexosamine and uronic acid.
- The largest and most ubiquitous is hyaluronan (hyaluronate or hyaluronic acid).
- Hyaluronan forms a viscous, pericellular network that binds a considerable amount of water (diffusion through connective tissue and in lubricating various organs and joints). This aids in lubricantion, viscosity and the gel-like texture. For example, in the umbilical cord there is a big amount of hyaluronic acid and the gelatinous nature of the umbilical cord comes from that.

GAGs

- All other GAGs are much smaller, sulfated, bound to proteins (as parts of proteoglycans).
- Major GAGs found in proteoglycans are dermatan sulfate, chondroitin sulfates, keratan sulfate, and heparan sulfate (different disaccharide units)
- Their high negative charge forces GAGs to an extended conformation and causes them to sequester cations as well as water.
- Molecules that carry negative charge are basophilic and will stain with hematoxylin, so they appear blue when stained in H&E. For example: cartilage matrix stains intensely basophilic, that's due to the negative charged GAGs that are present such as chondroitin sulfates.
- These features provide GAGs with space-filling, cushioning, and lubricant functions.

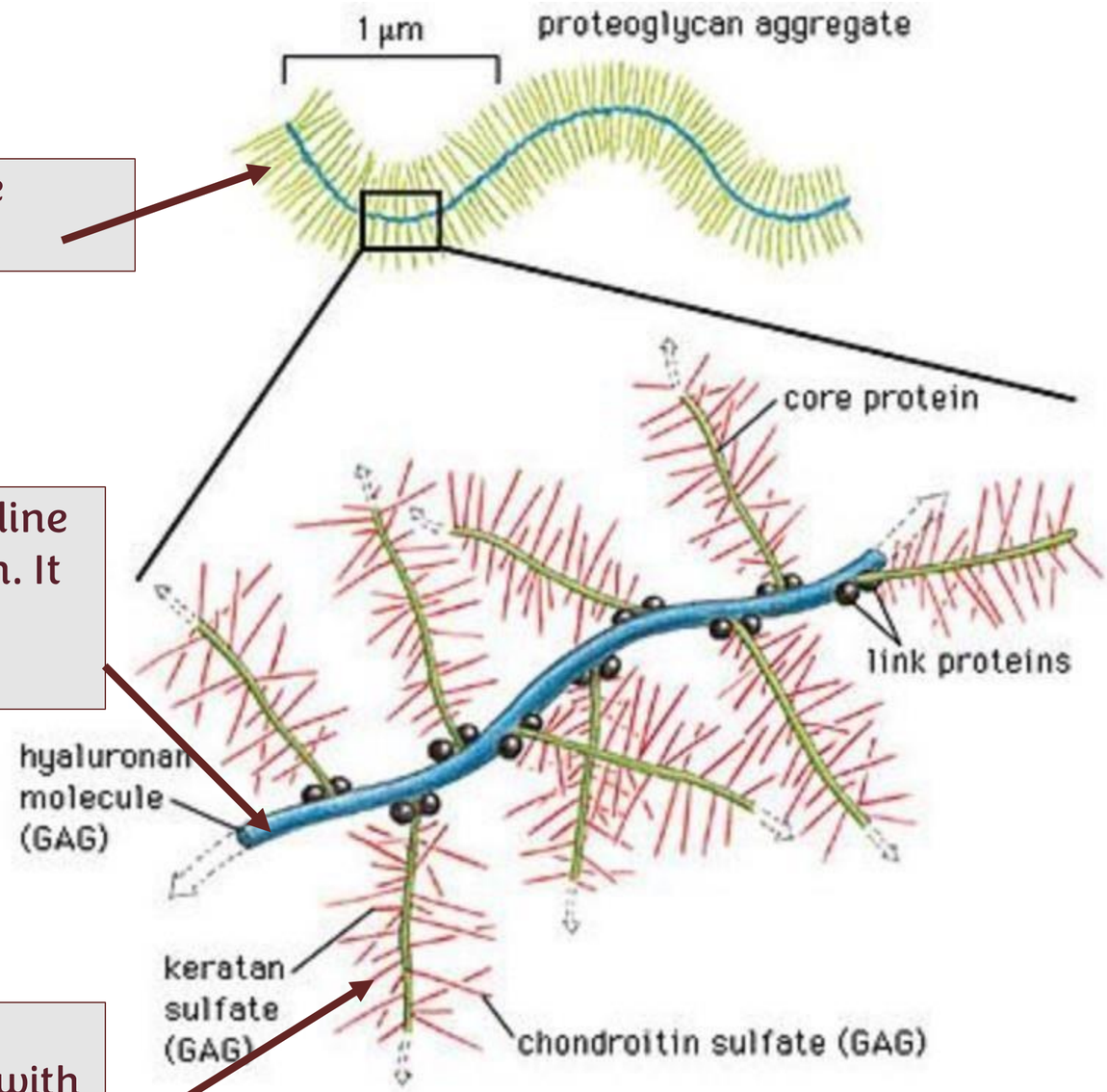
GAGs that form proteoglycans

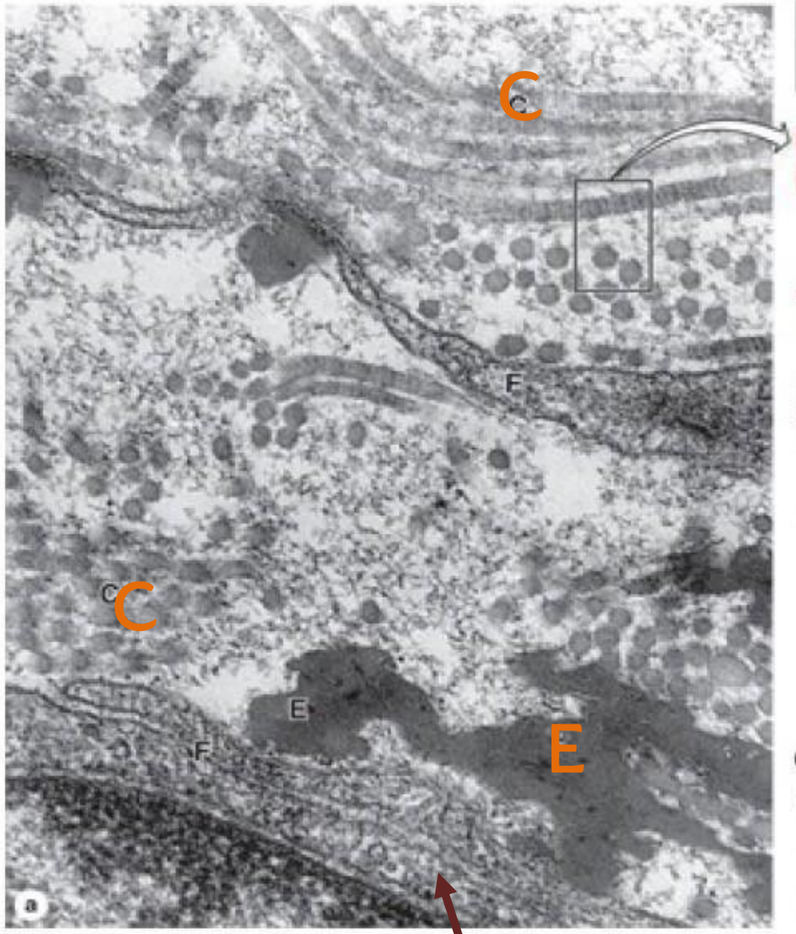
- Hyaluronic acid
- Chondroitin 4-sulfate
- Chondroitin 6-sulfate
- Dermantan sulfate
- Heparan sulfate
- Heparin
- Keratan sulfate

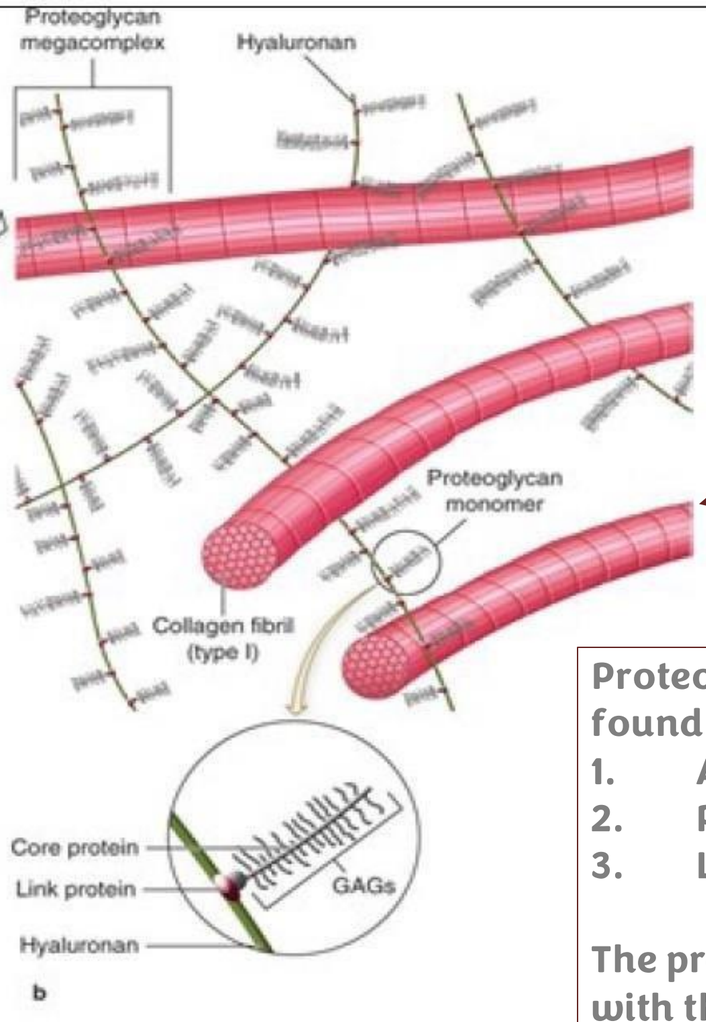
It looks like a worm with some structures coming out from it.

The huge core in the center (blue wavy line) is the biggest GAG which is hyaluronan. It acts as a backbone where it binds other smaller structures.

These small structures have a core protein (yellow) which is linked to the hyaluronan by linking proteins. This core binds with different GAGs depending on the type of tissue we have, for example: if we had a cartilage tissue the GAGs linked to the core protein will be rich with chondroitin sulfate .







This shows the ECM with its components, fibers and GAGs (ground substance).

Proteoglycan mega complexes are massive structures found in the ECM, they consist of :

1. A long hyaluronan backbone
2. Proteoglycans
3. Link proteins

The proteoglycans are attached to the long hyaluronan with the help of linking proteins, forming a large chain called a proteoglycan mega complex .

In the button left corner, this is a process of a fibroblast, (c) the perfectly rounded structures are collagen fibers cut cross-sectionally, and you can see them cut longitudinally aswell above. (e) irregular shaped structure , this is elastic fibers in relaxed form (not stretched). The ground substance is the filling between the cells and the fibers, all this tiny grayish space in between is the ground substance .

GAGs

Glycosaminoglycan	Repeating Disaccharides		Distribution	Electrostatic Interaction with Collagen
	Hexuronic Acid	Hexosamine		
Hyaluronic acid	D-glucuronic acid	D-glucosamine	Umbilical cord, synovial fluid, vitreous humor, cartilage	
Chondroitin 4-sulfate	D-glucuronic acid	D-galactosamine	Cartilage, bone, cornea, skin, notochord, aorta	High levels of interaction, mainly with collagen type II
Chondroitin 6-sulfate	D-glucuronic acid	D-galactosamine	Cartilage, umbilical cord, skin, aorta (media)	High levels of interaction, mainly with collagen type II
Dermatan sulfate	L-iduronic acid or D-glucuronic acid	D-galactosamine	Skin, tendon, aorta (adventitia)	Low levels of interaction, mainly with collagen type I
Heparan sulfate	D-glucuronic acid or L-iduronic acid	D-galactosamine	Aorta, lung, liver, basal laminae	Intermediate levels of interaction, mainly with collagen types III and IV
Keratan sulfate	D-galactose	D-glucosamine	Cartilage, nucleus pulposus, annulus fibrosus	None

READ ONLY: repeating Disaccharides.

MEMORIZE: types of GAGs and their distribution, you will know interaction with collagen when you know the structure of every connective tissue present here, so don't bother memorizing for now but you have to know it later on.

quiz on the lecture topics:

Press the start button

يلي بحل الامتحان صح يراجعنا ☺☺

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			

Additional Resources:

رسالة من الفريق العلمي:

اللهم علّمنا ما ينفعنا وانفعنا اللهم بما علمتنا واهدنا
واهدِ بينا يا رب العالمين

يقول النبي صلى الله عليه وسلم: "أحبُّ الناس إلى الله
أنفعهم للناس" 💙