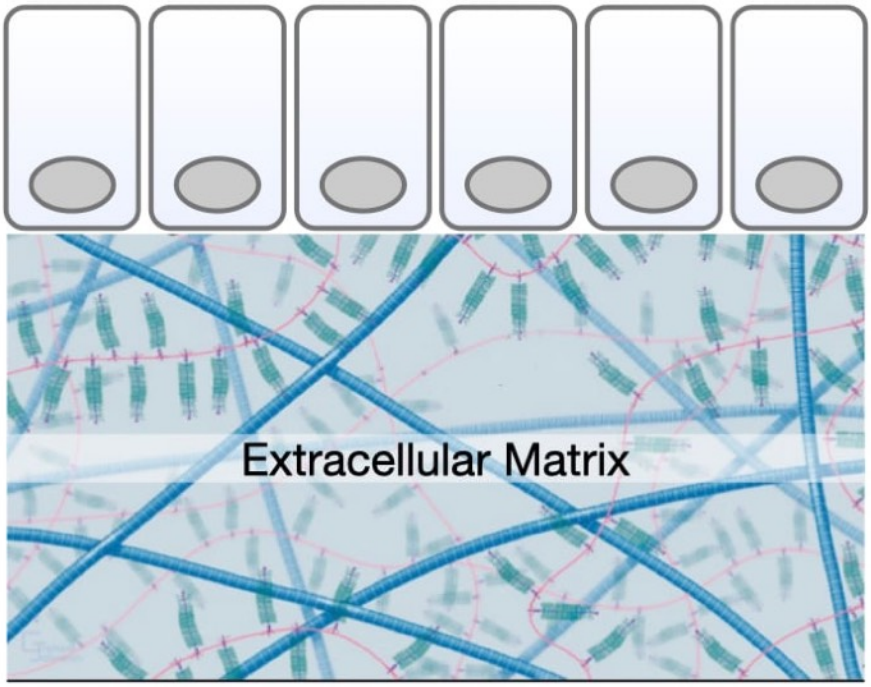
Extra Cellular Matrix

Scientific Foundations

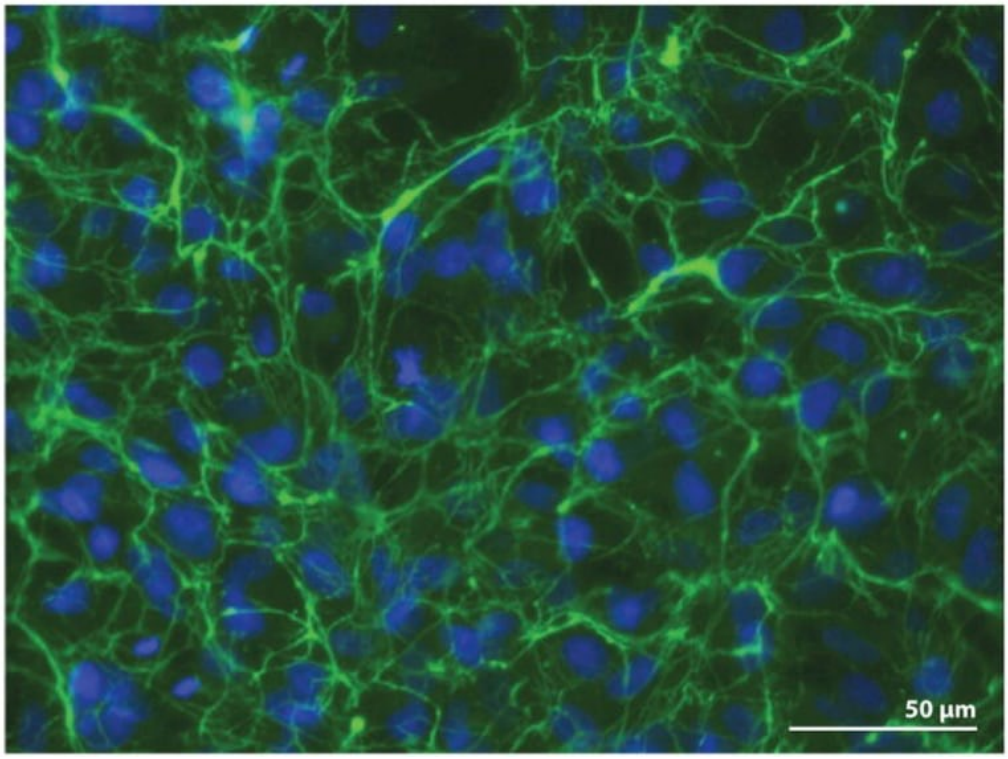
Functions of Extracellular Matrix

The extracellular matrix (ECM) is a collection of proteins, carbohydrates and fluid that perform a variety of functions in tissues. First, the ECM provides structural support to cells by allowing cells to attach to a common substrate. By connecting to the ECM, cells become integrated into a structural unit that has more robust mechanical properties than if cells were only attached to each other.

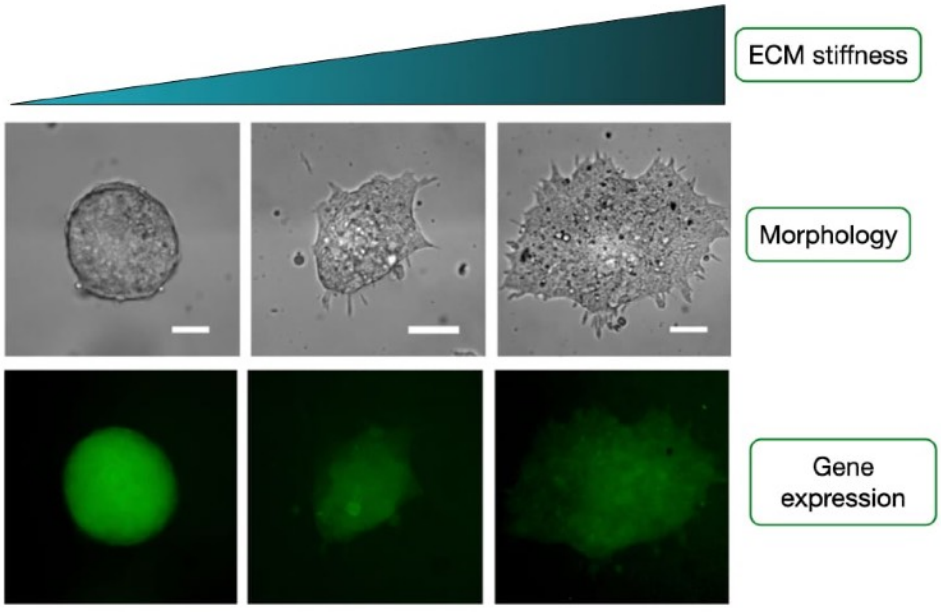


ECM provides structural support to cells.

In the image below, nuclei are stained in blue and the ECM is stained in green. Note how the ECM forms a network that surrounds the cells to provide structural support.

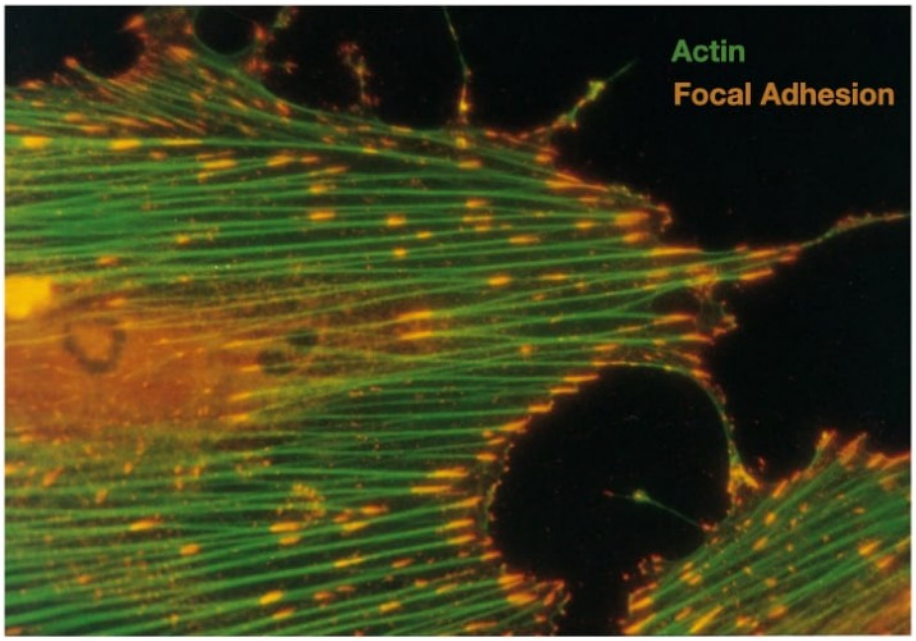


ECM also regulates activities within cells in two different ways. First, the mechanical properties of the ECM affect the behavior of cells. An ECM that is stiffer or more resistant to tension elicits different responses in cells compared to an ECM that is more easily deformed. For example, a stiff ECM can induce morphological changes in cells, trigger cell division or differentiation, and alter gene expression.



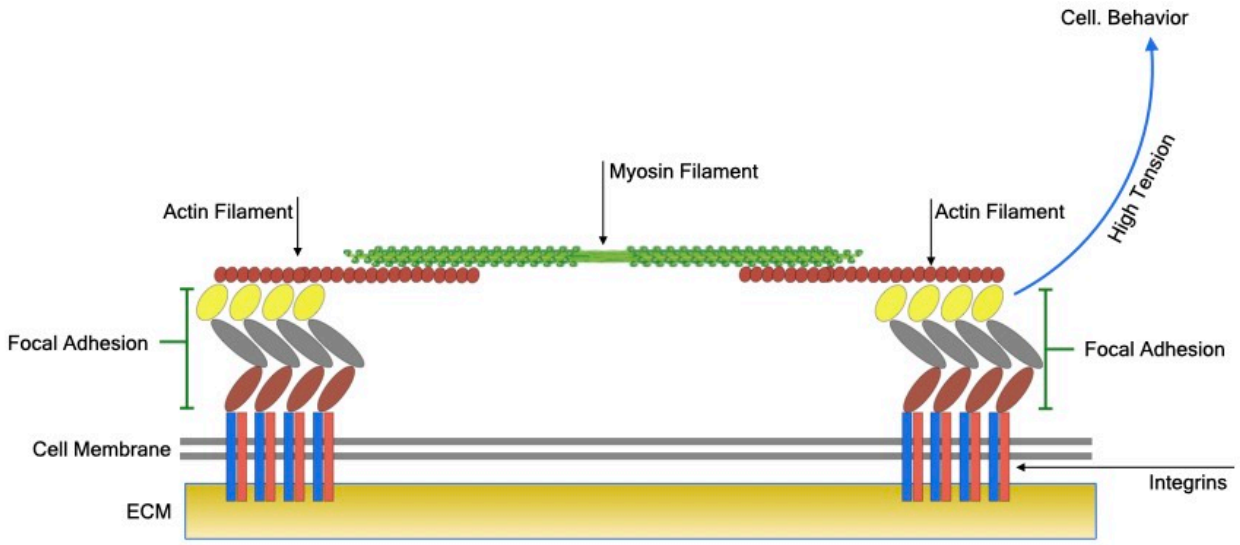
Extracellular matrix regulates cell behavior.

Cells sense the stiffness of the ECM through contacts they make with components of the ECM. These contacts often aggregate in structures called focal adhesions. Focal adhesions associate with actin filaments within the cytosol of cells.



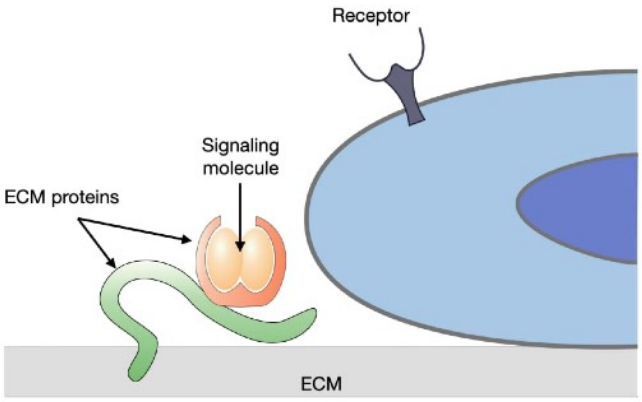
Cells connect to extracellular matrix at sites called focal adhesions.

Cells can generate tension on focal adhesions through actin and myosin filaments. Bipolar myosin filaments crosslink actin filaments that are attached to different focal adhesions. Focal adhesions are linked to the ECM via a set of integral membrane proteins in the cell membrane called integrins, which we'll discuss in detail later. When the myosin filaments are active, they pull on the actin filaments which in turn pull on the focal adhesions. If the focal adhesions are attached to an ECM that is stiff, the actin and myosin filaments can generate more tension on the focal adhesion because the stiffness of the ECM will resist movement of the focal adhesions. The higher tension on the focal adhesions initiates a signaling pathway which changes cell behavior. If focal adhesions are attached to a less stiff ECM, then the cell will not be able to generate as much internal tension and the signaling pathways will not be activated.



Cells measure ECM stiffness through tension generated by myosin and actin.

A second way that ECM controls cell behavior is by controlling the availability of signaling molecules. Hormones and other signaling molecules must diffuse through ECM to reach receptors on the surfaces of cells. Some of the components of the ECM bind to signaling molecules which slows their diffusion and in some cases, prevents them from binding to receptors on the surfaces of cells. Thus, ECM can regulate the concentration of signaling molecules that are available to cells and determine how tissues respond to signaling molecules.

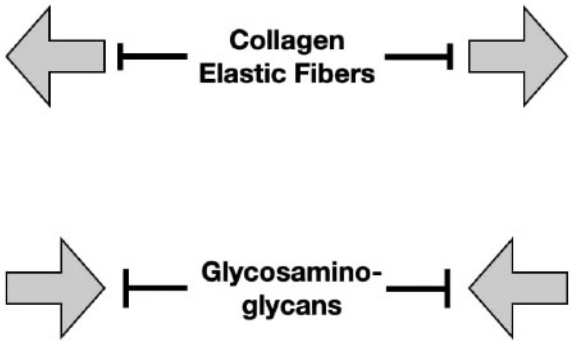


ECM regulates cell behavior by controlling the concentration of signaling molecules.

Components

There are three classes of protein components in extracellular matrix that largely determine its mechanical properties: collagen fibers, elastic fibers and glycoproteins. In general, these molecules either resist tensile and stretching forces or compressing forces. Collagen is the main component that resist tension. Elastic fibers also resist tension but behaves similar to rubber in that

it can be stretched and will recoil after the force is removed. On the other side are glycosaminoglycans that resist compressive forces.



Extracellular matrix resists tension and compression.

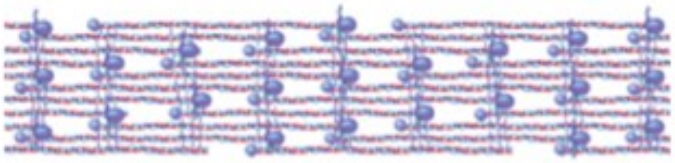
Collagen

Collagen is the most abundant class of proteins and pound for pound some are as strong as steel. There are several different types of collagens and their locations within the body varies. Most collagens, about 80-90% of total collagen, form fibers that provide the most mechanical strength. Aggregation and lateral interactions between the individual fibers increase the mechanical strength.

There are over twenty different types of collagen but the most abundant and medically significant are types I - IV. Types I - III form fibers which gives them greater mechanical strength and ability to resist tension. We'll discuss these collagens and their tissue location in more detail when we cover connective tissue. Type IV forms a branched network which allows it to form a sheet-like structure. We'll discuss type IV collagen in more detail when we cover epithelia.

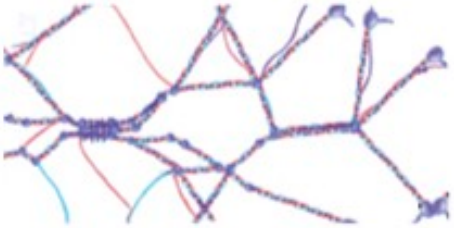
Fibrillar:

- Type I: Bone, ligament, skin, tendon
- Type II: Collagen
- Type III: Reticular fibers



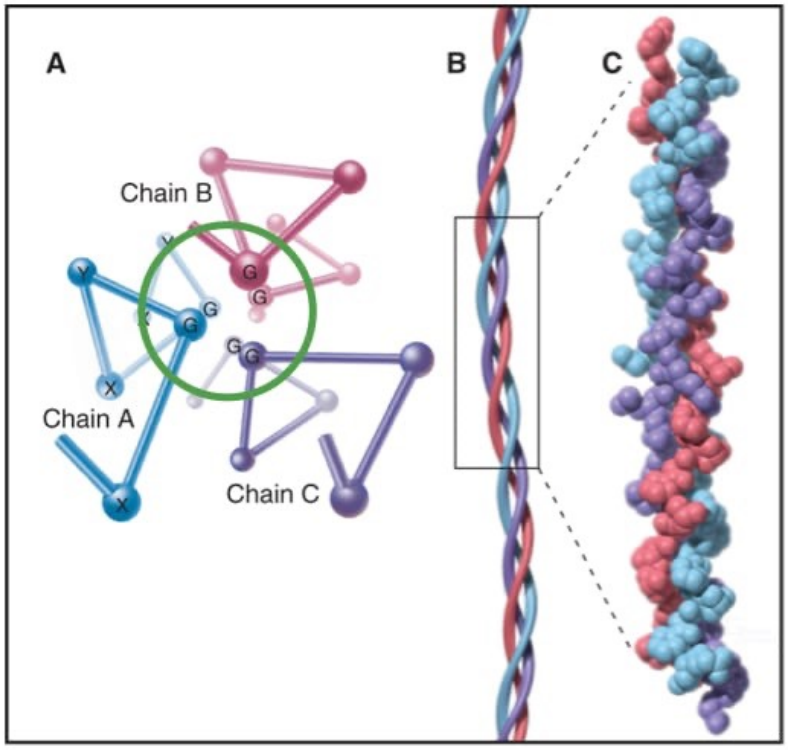
Network

- Type IV: Basement membrane



Collagens are a large family of proteins that form fibers or networks.

Type I - IV collagens are composed of three polypeptide chains. Each of these polypeptides can be over 1000 amino acids giving the trimer an overall length of 300 nm and a width of 1.5 nm. A collagen trimer can be composed of polypeptides encoded by different genes (Type I and IV) or polypeptides from the same gene (Type II and III). The polypeptides form alpha-helices that wrap around each other to form coiled coil interactions. The sequence for most of the length of a collagen polypeptide is a repeat of 3 amino acids: glycine and usually proline and lysine. Glycine which is the smallest amino acid allows for tight packing of the polypeptides in the trimer. Note the extensive lateral interactions that give the structure its mechanical strength.



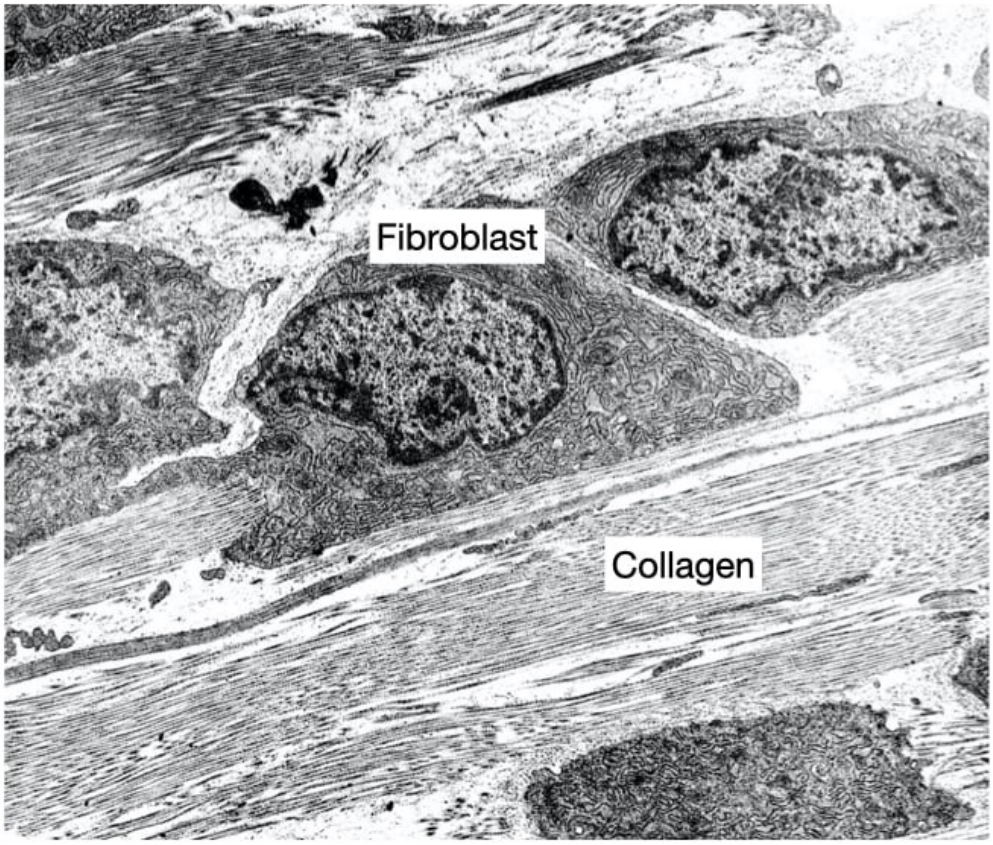
Three collagen polypeptides associate to form rope-like structures.

Each type of collagen is encoded by one or more genes:

- Type I Collagen: COL1A1 and COL1A2
- Type II Collagen: COL2A1
- Type III Collagen: COL3A1
- Type IV Collagen: COL4A1 - COL4A6

Thus, type I collagen trimers are composed of a mix of A1 and A2 protein.

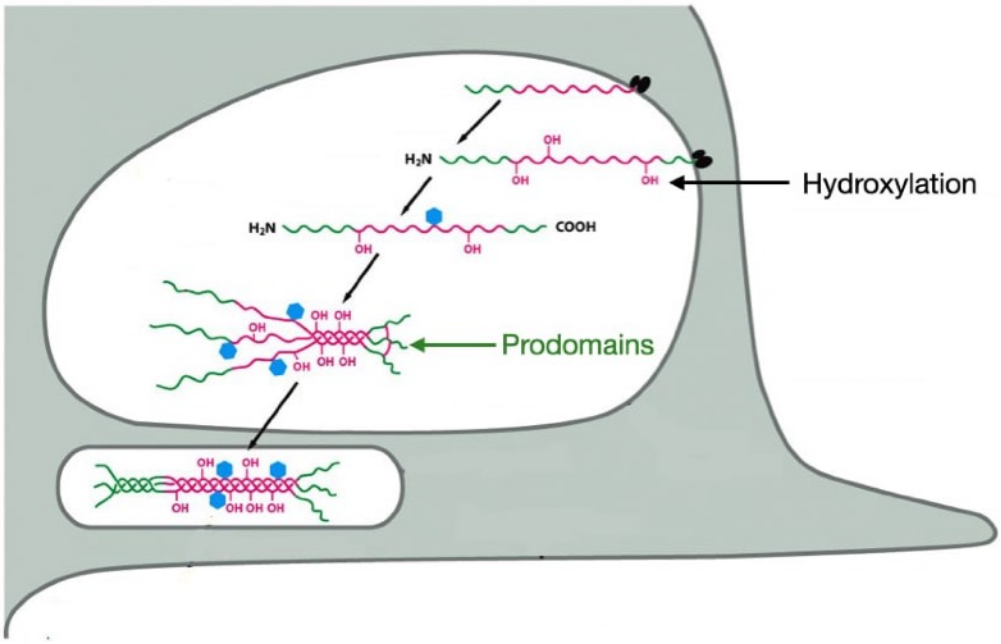
Many different types of cells can synthesize collagen but one of the most prominent in extracellular matrix is the fibroblast shown here in an electron micrograph. We'l encounter fibroblasts again when we discuss specific tissues and organs.



Fibroblasts synthesize and process collagen.

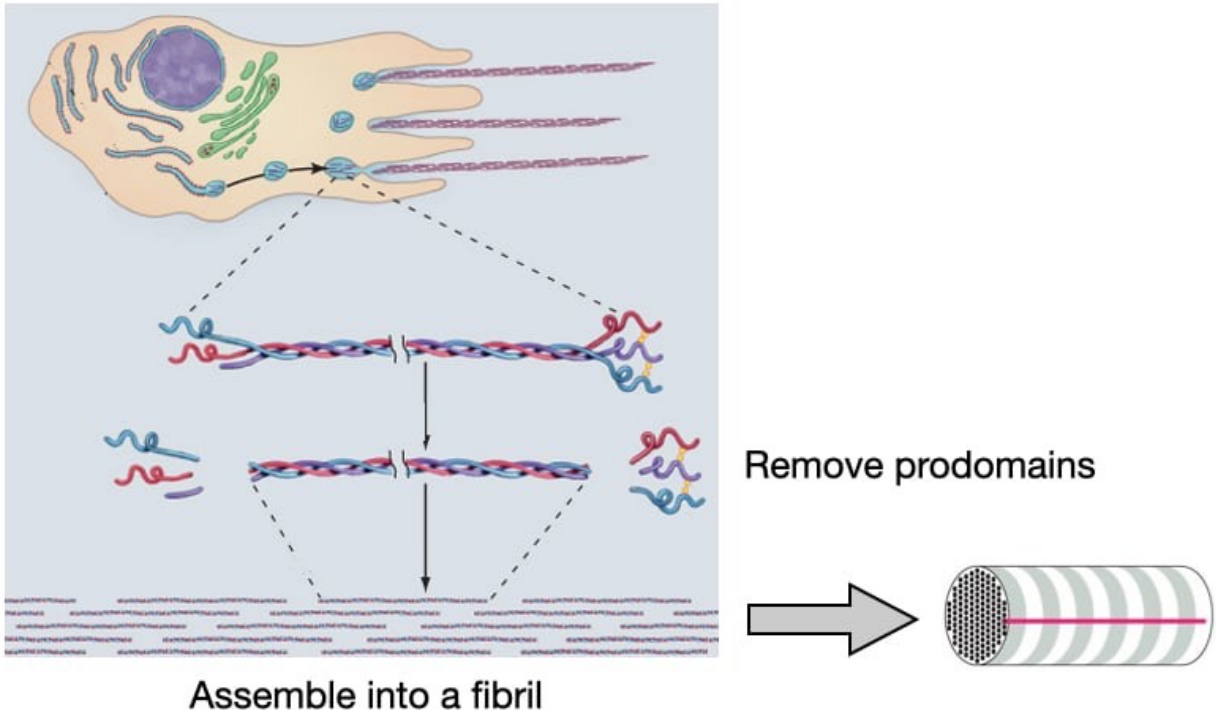
The synthesis of collagen polypeptides in fibroblasts involves several key steps that allow them to assembly into collagen fibrils. Because collagen is a secreted protein, it is synthesized on ER-bound ribosomes and translocated across the ER membrane during translation. One important feature of collagen is that it contains extra sequence at its N and C-termini called prodomains. These prevent collagen trimers from assembling into fibrils inside the cell which would be catastrophic for the cell.

Collagen undergœs two important modifications in the ER. First, certain prolines and lysines are hydroxylated. These modifications will allow for assembly into trimers and covalent crosslinks between collagen trimers outside the cell. Second, disulfide bonds between collagen polypeptides mediates their assembly into trimers by facilitating interaction between correct collagen proteins.



Fibroblasts synthesize and secrete collagen via secretory pathway.

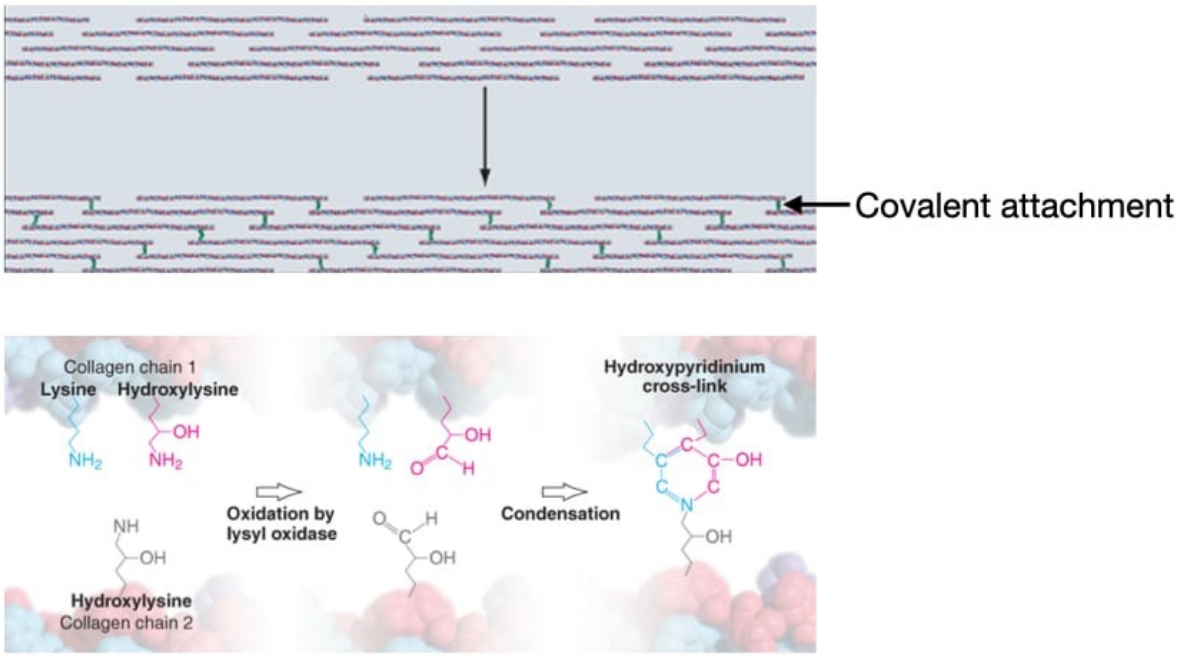
Once secreted, the prodomains are removed from the trimer by proteases that reside outside fibroblasts to produce the mature collagen trimer. Collagen trimers then self-assemble into fibrils through an entropy-driven process (dœs not require input of energy).



Removal of prodomains outside the cell allows collagen to assemble into fibrils.

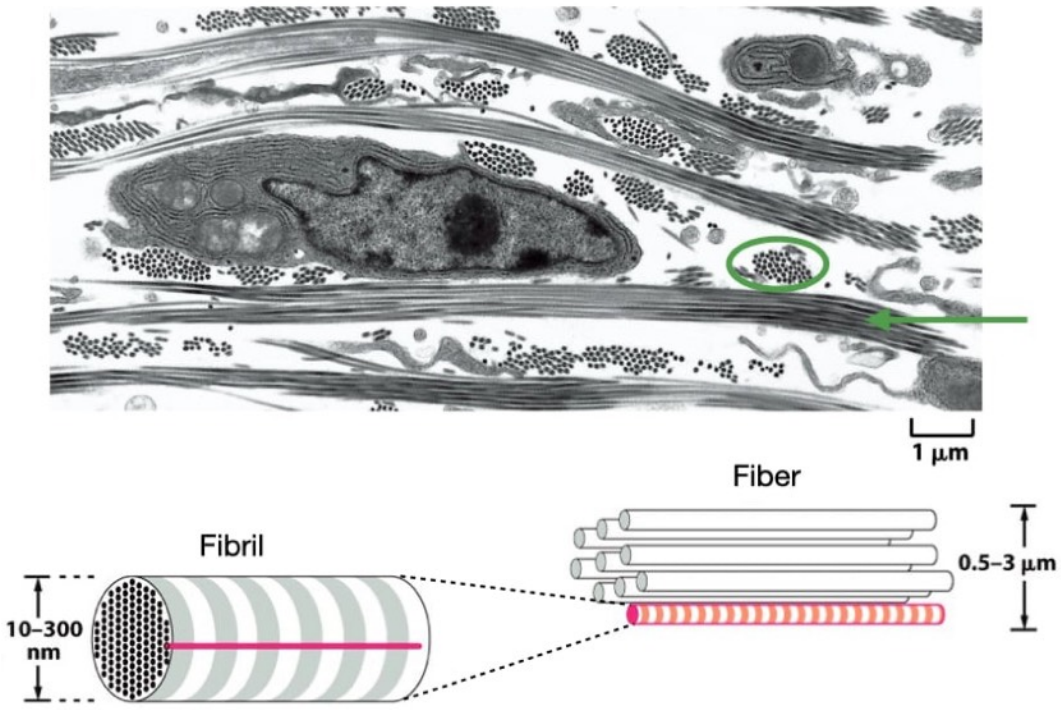
The interactions between collagen trimers allow them to assemble into fibrils but the interactions are insufficient to account for collagen's ability to resist tension. Covalent crosslinks between

adjacent trimers generates a stronger interaction between trimers. Lysyl oxidase catalyzes a reaction that generates covalent bonds between hydroxlysines in adjacent trimers. Mutations that affect hydroxylation of lysines weakens collagen.



Lysyl oxidase catalyzes covalent bonds between hydroxylated amino acids in adjacent trimers.

Some collagen fibrils (type I) then aggregate into large bundles called fibers. Note in the image below collagen fibers are shown in cross-section (green circle) and longitudinally (green arrow). In cross-section each dot is a collagen fibril and the collection of fibrils composes a fiber.



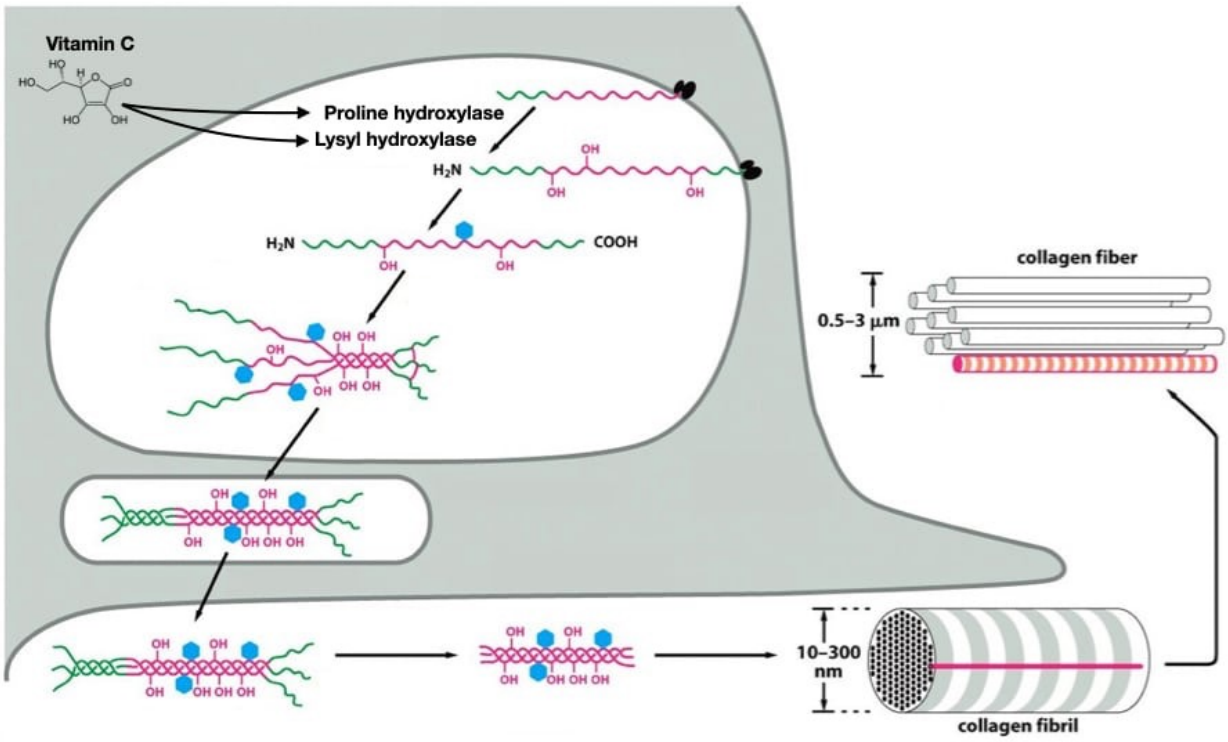
Type I collagen fibrils aggregate to form fibers.

This gives type I collagen three levels of lateral interactions:

- Trimer - assembles inside fibroblasts.
- Fibrils - aggregation of trimers outside of fibroblasts and crosslinked by lysyl oxidase..
- Fibers - aggregation of fibrils.

The extensive interactions are in part what make type I so effective at resisting tension.

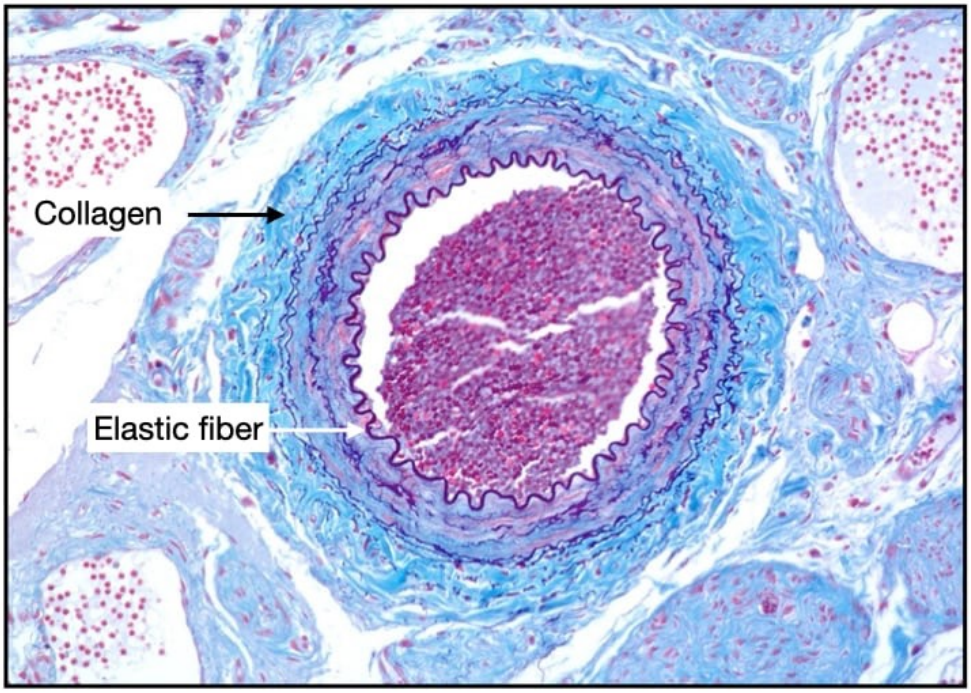
The proper assembly of collagen is dependent upon two enzymes that act immediately after collagen is synthesized in the ER. Proline and lysyl hydroxylases convert proline and lysine into hydroxylated versions. Hydroxylated proline facilitates packing of individual collagen polypeptides into a trimer and hydroxylated lysine is required to generate covalent attachments between trimers.. Both enzymes require vitamin C as a cofactor and people who don't consume enough vitamin C will produce collagen that lacks hydroxylated lysines and prolines. Because these trimers cannot be crosslinked, the collagen fibrils will be weaker leading to tissues that are more prone to damage.



Vitamin C is a critical cofactor in the assembly of collagen fibrils.

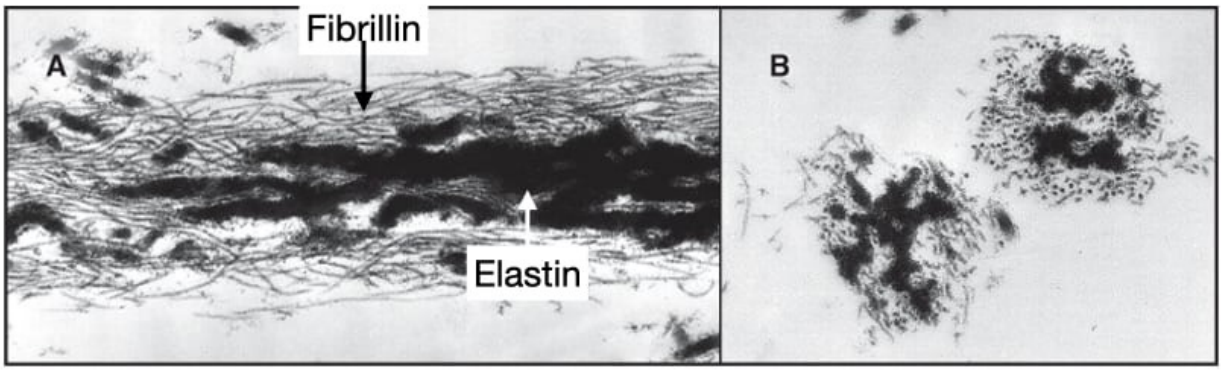
Elastic Fibers

Next, we will examine elastic fibers that are often found enmeshed with collagen fibers as shown in this cross section of an artery. The elastic fibers stain dark blue and appear as a wavy line whereas the collagen stains light blue. Elastic fibers have different mechanical properties from collagen. They allow for stretching of tissues under external force, but generate a recoil force when the external force is removed. Elastic fibers are prominent in the walls of arteries especially the aorta. The elastic fibers stretch to allow the aorta to accommodate a large volume of blood during systole. When the pressure drops during diastole, the fibers recoil pushing blood into the circulatory system. Because of elastic fibers a consistent blood pressure is generated in the circulatory system, even though the pumping of the heart delivers blood in a pulsatile fashion.



Elastic fibers allow tissues to stretch and recoil.

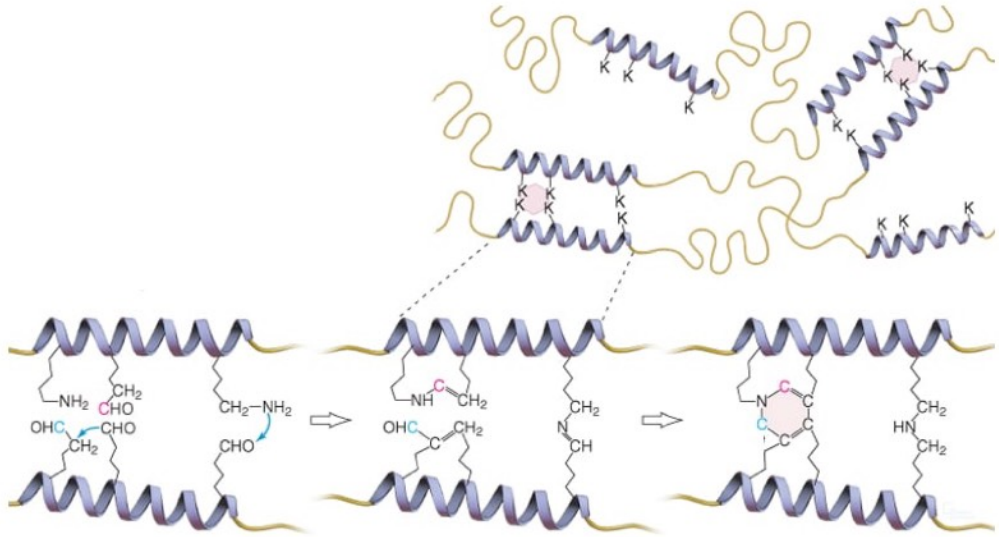
Elastic fibers are a composite material composed of two primary components: elastin and fibrillin. Both are synthesized by fibroblasts and other cells and are secreted into the surrounding tissue where they assemble into elastic fibers. Fibrillin fibers are thin and arranged in more or less parallel arrays. They are required for correct assembly of elastic fibers. Elastin appears as an amorphous substance. Elastin is what gives elastic fibers its characteristic mechanical properties of stretching and recoiling.



Elastic fibers are a composite of elastin and fibrillin.

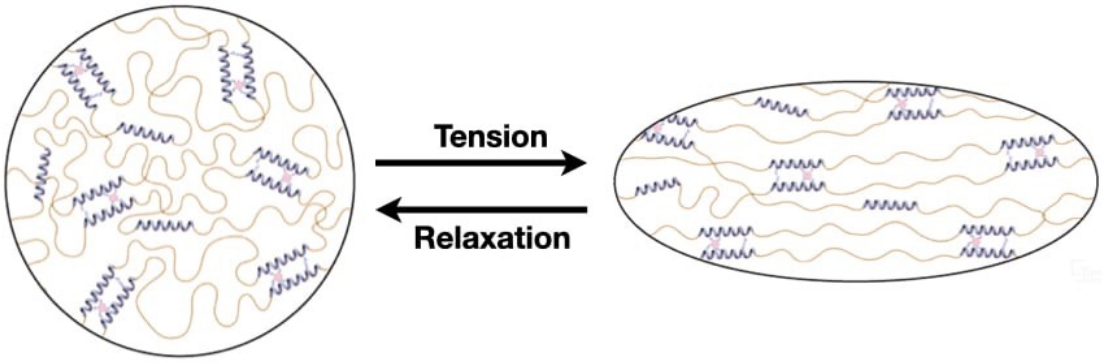
Elastin is the main structural component of elastic fibers. Compared to collagen, elastin is largely unstructured which gives it that amorphous appearance in electron micrographs. Elastin has a hydrophobic domain which mostly lacks structure and an alpha-helical domain. Lysyl oxidase

generates covalent attachments between lysines in the alpha-helical domain of adjacent elastin polypeptides. This generates a network of interconnected elastin proteins.



Elastin is an unstructured protein that is crosslinked into networks.

The unstructured nature of elastin accounts for its ability to stretch and recoil. Relaxed fibers largely unstructured and disordered. The hydrophobic domains cluster to avoid water. Tension stretches elastin generating order in hydrophobic domains. When tension is removed, elastin returns to its lower energy state of disordered fibers, causing the elastin network to recoil. The relaxation of elastic fibers dœs not require input of energy but is due to the transition from a high energy state to a low energy state.



Tension generates order in elastin networks that provides energy for recoil.

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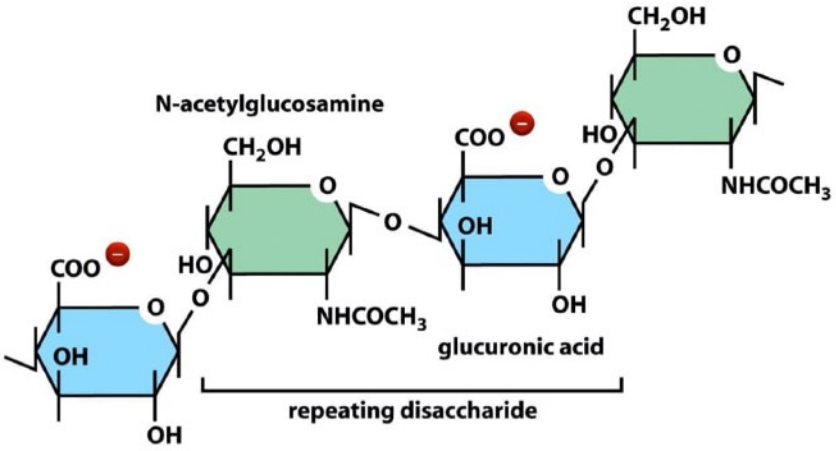
Glycosaminoglycans

Extracellular matrix not only resist tensile or pulling forces but also resist compression. The primary component in ECM that responds to compression are a family of molecules called glycosaminoglycans (GAGs). GAGs resist compression by occupying a large volume and retaining water within that space. This is similar to how a plastic bottle filled with water resists compression. An air-filled bottle collapses under applied force because the force expels the air from the bottle. In contrast, a bottle filled with water that is sealed so that it retains water, resists compression from an outside force.

Glycosaminoglycans (GAGs) in connective tissue resist compression by retaining water.

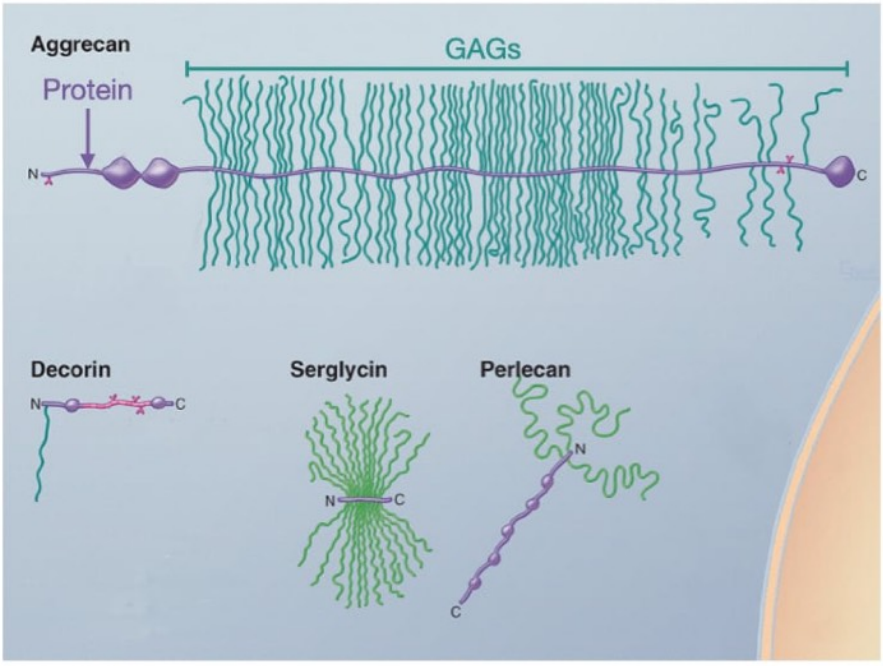
Glycosaminoglycans (GAGs) are the main component that retains water to resist compression. The base component of GAGs is a disaccharide of two different sugars. These disaccharides are joined into polymers that can contain thousands of disaccharides. The sugars that compose the disaccharides differ between GAGs, but a feature they share is a negative charge on one of the sugars. Thus, GAGs are long, negatively-charged polymers. The negative strong charge in GAGs attracts sodium which through osmosis attracts and retains large amounts of water within GAGs.



Glycosaminoglycans are long chains of repeating disaccharides.

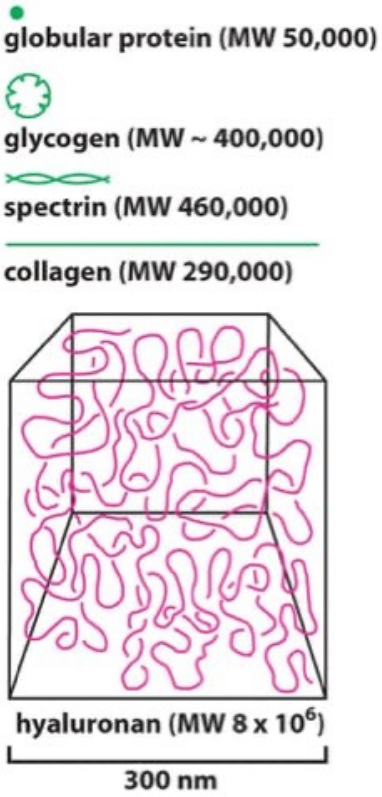
Proteoglycans

GAGs come in two different types. Proteoglycans are a single polypeptide that contains a few or many GAGs attached as side chains. Addition of GAGs to the protein occurs in the secretory pathway. The disaccharide side-chains are linked to proteins in the ER and Golgi. Proteoglycans include proteins of different lengths and different number sand types of covalently attached GAGs. Fibroblasts and other cells secrete proteoglycans through the secretory pathway.



Proteoglycans are single polypeptide with several attached glycosaminoglycans.

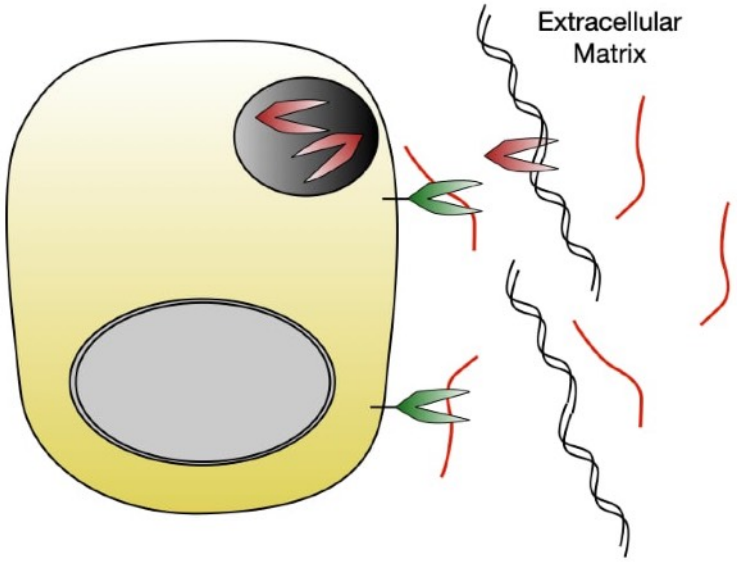
Hyaluronan is another glycosaminoglycan but is unique because it is not attached to a proteins but exists as a free polysaccharide. Hyaluronan can contain up to 25000 repeats of a disaccharide and can reach a length of 20 μm, the size of an average cell. Hyaluronan lacks the structure of most proteins and contains many regions of that form random, flexible coils. Remember that the sugars in hyaluronan are negatively charged and repel each other. That generates a lot of space within the hyaluronan and allows it to occupy an incredibly large volume. In addition, hyaluronan like other GAGs bind and retains water which enables it to resist compression.



Hyaluronan is a long polymer of dissaccharides that occupies a large volume.

Digestions of Extracellular Matrix

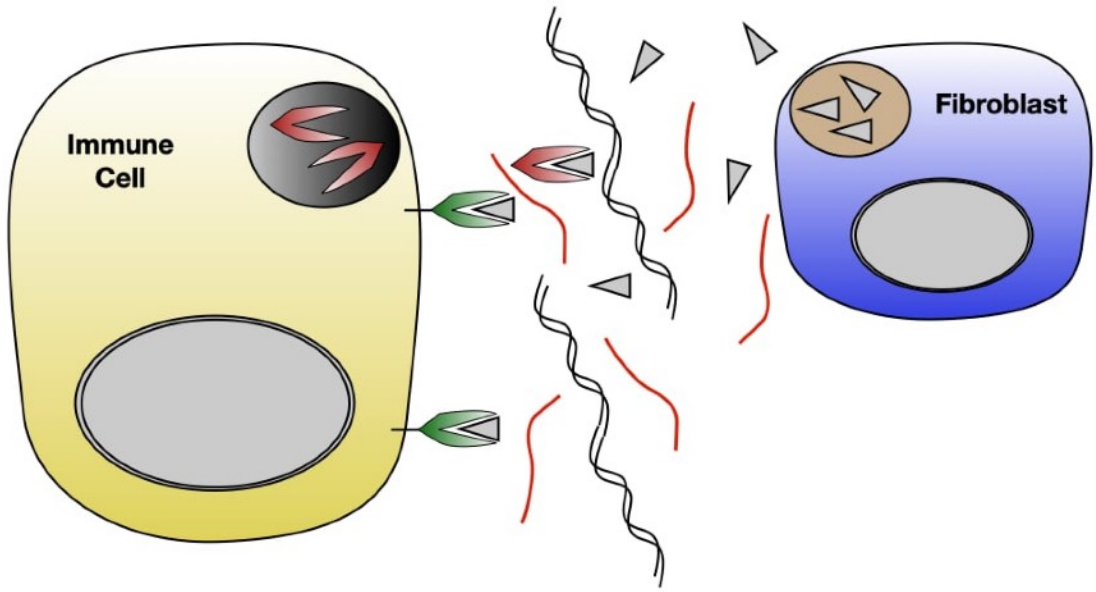
Like many structures in the body, the components of the extracellular matrix must be replaced over time due to damage to the proteins that compose the ECM. Specific enzymes called matrix metalloproteinases (MMPs) digest the proteins in the ECM. MMPs can be secreted into the extracellular space or expressed as an integral membrane protein in the cell membrane. Fibroblasts synthesize new protein fibers to replace the digested components.



Some cells produce enzymes that digest components of extracellular matrix.

Digestion of the ECM also plays an important role in certain biological processes including development and immune responses.

Fibroblasts limit the extent of digestion of ECM by secreting proteins called tissue inhibitors of MMPs (TIMPs). TIMPs inhibit the activity to MMPs to ensure that the enzymes don't degrade too much of the ECM which would impair the structure and function of cells in a tissue or organ. A balance between the amount of matrix metalloproteinases and inhibitors of matrix metalloproteinases is essential to maintain the integrity of the ECM while allowing for necessary biological process. Disease can arise when the balance between MMPs and TIMPs is significantly shifted.



Fibroblasts secrete tissue inhibitors of metalloproteases (TIMPs) to limit digestion.