

CONNECTIVE TISSUE (I) PROPER

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Reading: Gartner & Hiatt, Chapter 3

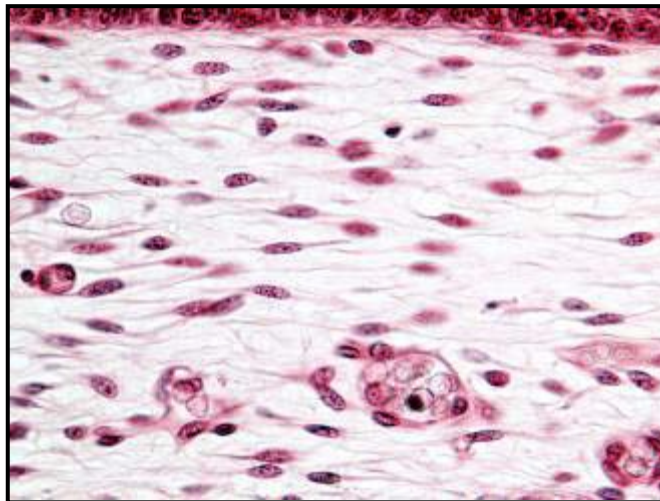
Objectives:

- List components of ground substance.
- List major fiber types found in connective tissue matrix.
- List 5 types of collagen and their respective distributions.
- Identify the cell types of connective tissue proper, their origins, and major functions.
- Recognize and classify 3 types of adult connective tissue proper.
- Review the composition of the basement membrane.

Key Words: Mesenchyme, fibroblast, macrophage, Kupffer cell, plasma cell, eosinophil, mast cell, collagen, ground substance, basement membrane

I. COMPOSITION OF CONNECTIVE TISSUE

- A. Composed of **cells** embedded in an **extracellular matrix (ECM)**
 1. Composed of ground substance, fibers, and tissue fluid.
 2. The variation in the cells and components of the ECM is responsible for the different types of connective tissue throughout the body.
- B. Functions in a wide variety of supportive and protective roles.



Connective Tissue (G&H, p. 55)

II. GROUND SUBSTANCE

- A. A colorless, transparent mixture of proteins found between the cells and fibers of connective tissue.
 1. High viscosity contributes to its role as a lubricant and a physical barrier to penetration by bacteria.
 2. Composed of glycosaminoglycans (GAGs), proteoglycans, and glycoproteins.

B. Glycosaminoglycans

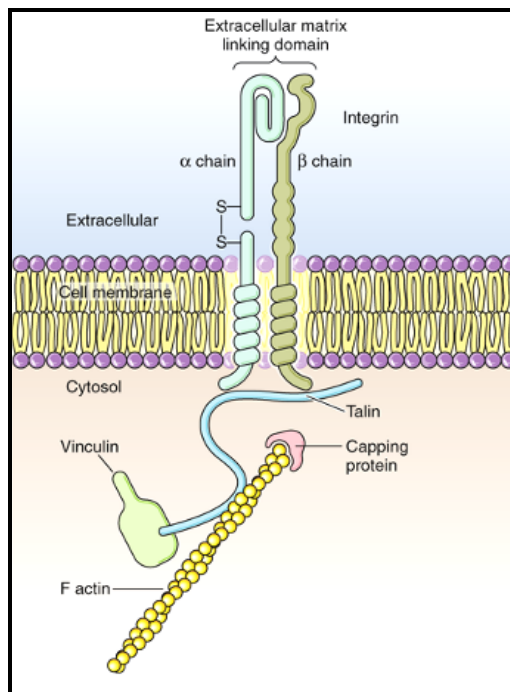
1. Long, unbranched polysaccharide chains consisting of repeating disaccharide units. All are sulfated, except hyaluronic acid, and have a strong negative charge.
2. Hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparan sulfate, and keratan sulfate.

C. Proteoglycans

1. All GAGs, except those composed of hyaluronic acid, can be covalently bound to a protein core to form **proteoglycans**. These molecules are 80-90% carbohydrate by weight and attract water to form a gel.
2. In addition to giving support to the connective tissue matrix, they act as binding sites for various growth factors and signaling molecules.

D. Glycoproteins also contain a protein core and carbohydrates, however the protein component predominates and the carbohydrate moiety is branched rather than linear.

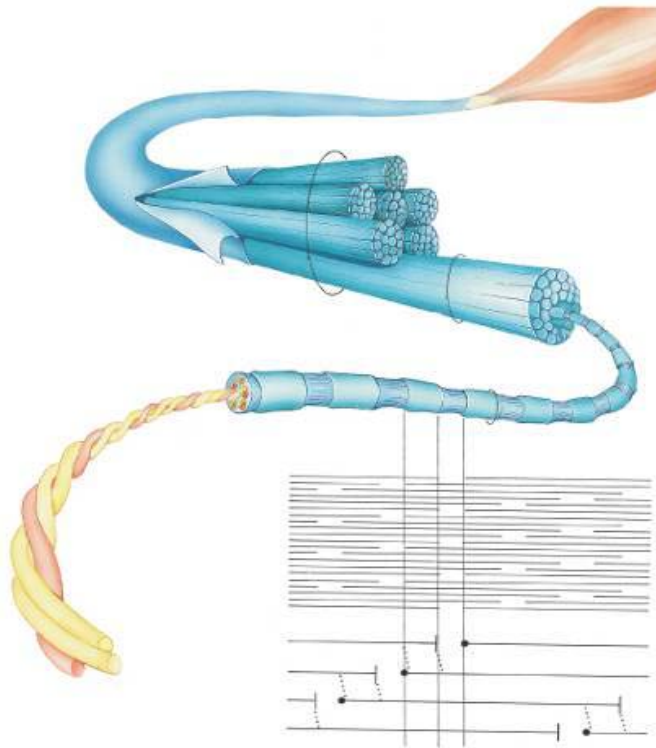
1. Function in the adhesion of cells to each other and to the fibers of the extracellular matrix
 - a. **Fibronectin** is made by fibroblasts and mediates normal cell adhesion and migration.
 - b. **Laminin** is made by epithelial cells and mediates adhesion of these cells to the underlying basement membrane.
2. **Integrins** – transmembrane proteins on cells that serve as receptor sites for the glycoproteins. One end binds to proteins in the ECM; the other end attaches to actin microfilaments within the cell. This process is mediated by intracellular proteins (**paxilin, vinculin, talin**).



III. FIBERS OF CONNECTIVE TISSUE

- A. **Collagen Fibers** – Composed of the protein “collagen” and are important components of tissues requiring rigidity, flexibility, and strength.
1. Synthesized by a wide variety of cells, including fibroblasts, chondroblasts, osteoblasts, smooth muscle, endothelial and epithelial cells. Is the most abundant protein in the body.
 2. Synthesis (intracellular and extracellular components):
In the RER, polypeptide chains known as **procollagen** are formed. These chains are subsequently transported outside the cell, and cleaved by proteases to yield **tropocollagen**. Tropocollagen molecules then spontaneously assemble into **collagen fibrils**. Fibrils are very long, thin structures that demonstrate a characteristic banding pattern due to the overlapping of the tropocollagen subunits. Fibrils cannot be seen with light microscopy (LM), only with TEM. Fibrils further crosslink to form **collagen fibers**; these can be seen with LM, especially when they form larger collagen **bundles** (appear eosinophilic).

Collagen Synthesis (Gartner & Hiatt, p. 52)



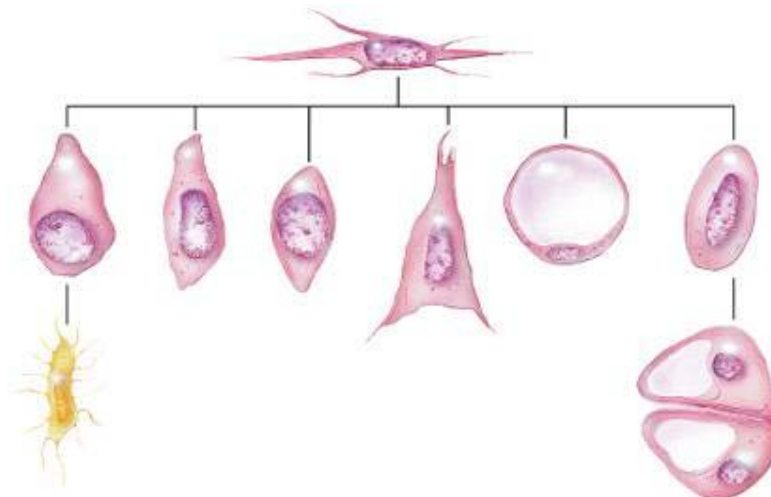
3. At least 25 different types of collagen have been identified and they are classified according to their structure and function. The following 5 types are of greatest importance:

Major Types of Collagen

Collagen Type	Appearance	Microscopy	Distribution	Main Function
I	Fibers, bundles	LM	CT proper, dermis, bone, dentin, tendon, ligaments, fibrocartilage	Resistance to force, tension and stretch
II	Fibrils	TEM	Cartilage, intervertebral disc	Resistance to pressure
III	Fibrils, very thin fibers (Reticular fibers)	Special stains (argyrophilic)	Loose CT (along with Type I collagen), reticular tissue (organ stroma, perivascular CT), lamina reticularis	Maintenance of structure in expansible organs and tissues
IV	Not visible	Special stains	Basal lamina (lamina densa)	Support of delicate structures
VII	Not visible	TEM	Dermal-Epidermal junctions	Attach lamina densa to underlying connective tissue (lamina reticularis)

B. **Elastic Fibers** – Composed of the structural protein **elastin**, and the glycoprotein **fibrillin** that organizes elastin into fibers. The presence of elastin allows the fibers to stretch in response to tension (unlike collagen fibers). Elastin contains 2 unique amino acids, desmosine and isodesmosine.

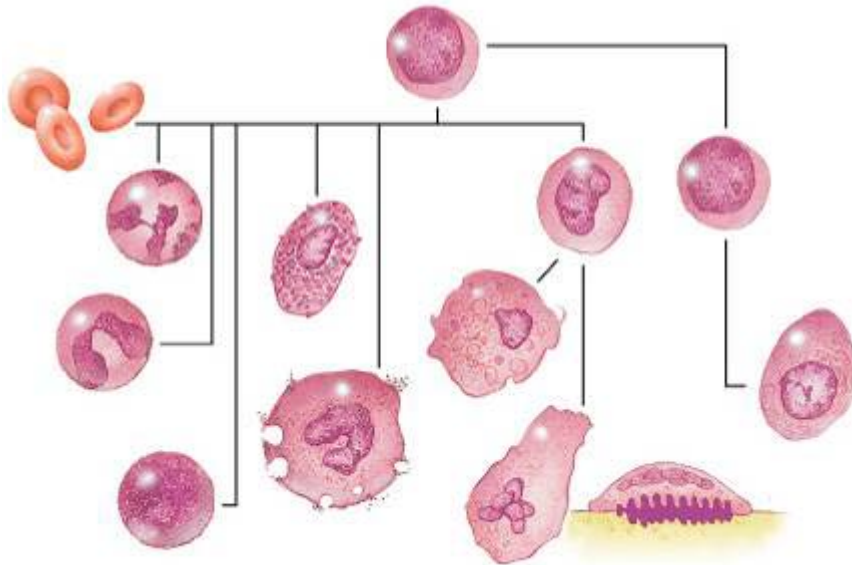
- Often interwoven with collagen fibers and found in elastic vessels like the aorta and large arteries. Also found in the lungs, skin, and elastic cartilage. In the walls of larger blood vessels, elastin forms sheets known as **elastic laminae**.



CT Cell Differentiation (G&H, p. 53)

IV. CELLS OF CONNECTIVE TISSUE

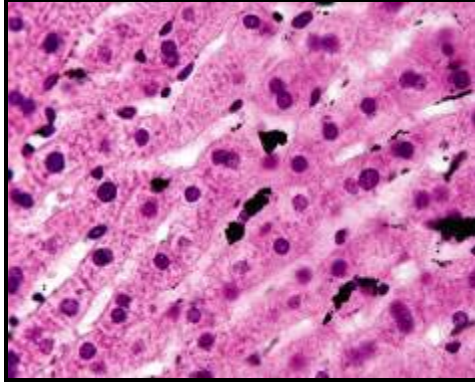
- A. **Fibroblast** – Responsible for formation and maintenance of all types of fibers and ground substance. Two morphologically distinct types:
1. **Active** fibroblast has a large oval nucleus, prominent nucleolus, and abundant eosinophilic cytoplasm.
 2. **Resting** fibroblast (referred to, incorrectly, as a **fibrocyte** in older books) is smaller, spindle-shaped, and has a slender elongated dark nucleus. Cytoplasm is indistinct. In dense CT, the nuclei tend to be oriented in the direction of the collagen fibers.



Stem Cell Differentiation (G&H, p. 53)

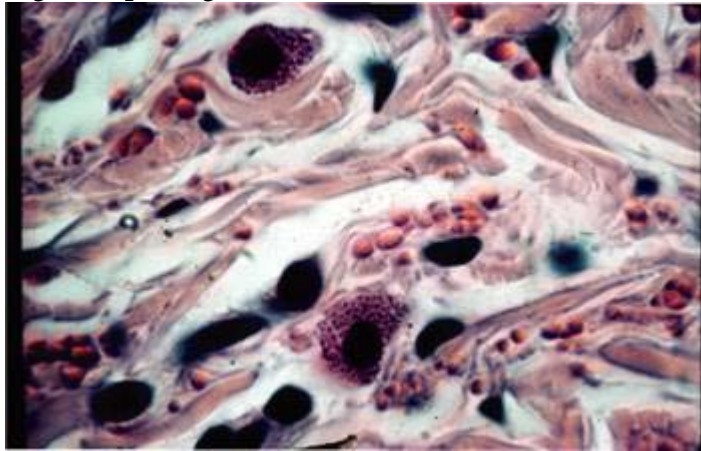
- B. **Macrophage** – These cells make up the **mononuclear phagocyte system (MPS)** and are present in most organs of the body. Derived from stem cells in the bone marrow that enter the blood stream as **monocytes**. Monocytes subsequently make their way into the connective tissues and mature into macrophages.
1. Major functions: ingestion and digestion of foreign substances, bacteria, and old cells; storage of iron in the liver; release of inflammatory mediators; and antigen recognition and processing.

CELL NAME	LOCATION
Macrophage	CT proper, lung, spleen, lymph node
Kupffer cell	Liver
Dust cell	Lung
Microglia cell	Central nervous system
Osteoclast	Bone
Langerhans cell	Skin



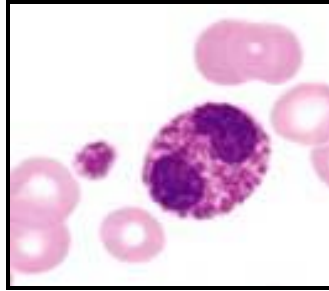
Macrophages (G&H, p.59)

- C. **Mast Cell** – Participates in inflammatory and allergic responses through the release of mediators such as heparin, histamine, leukotrienes, and eosinophil chemotactic factor of anaphylaxis (ECF-A). Also originate from stem cells in the bone marrow. Are most abundant in the skin, the GI and respiratory tracts, and around blood vessels.
1. Cytoplasm contains numerous secretory **granules**. Granules are not visible with H&E, but show up well with toluidine blue (a blue basic dye) as large **basophilic** granules.



Mast Cell (G&H, p. 59)

- D. **Eosinophil** – Considered a transient cell in CT since under normal conditions, it is seen only in the intestinal tract. It is not routinely be seen in other CT sites. Involved in parasitic infections and allergic reactions.
1. Cytoplasm is filled with large electron-dense granules that contain **major basic protein**, which is toxic to parasites. These granules appear bright orange-red with H&E stains and account for the **eosinophilia** of the cytoplasm. The nucleus is typically **bi-lobed**.



Eosinophil (G&H, p. 95)

- E. **Lymphocyte** – Involved in the immune response and are found in connective tissues throughout the body. There are 2 types, **B lymphocytes** and **T lymphocytes**; they cannot be differentiated from each other in tissue without the use of special stains.
1. Nucleus is round or slightly oval, heterochromatic, and takes up the majority of the cell. A thin rim of blue cytoplasm may be visible. Therefore, in tissues these cells are typically seen as apparently “naked nuclei” without visible cytoplasm.
 2. Predominate in the loose connective tissue of the respiratory and GI tracts where they serve to protect the body against invasion by bacteria and foreign particles.



Lymphocyte (G&H, p. 95)

- F. **Plasma Cell** – Derived from **B-lymphocytes** and function in the synthesis of antibodies (a.k.a. immunoglobulins). Therefore, they are most numerous in the sites prone to invasion by bacteria and foreign substances.
1. Large oval cells with an eccentrically placed nucleus. The cytoplasm stains basophilic due to the large amount of RER. Prominent **Golgi** are situated near the nucleus, resulting in a light-staining area (sometimes) of the cytoplasm known as a “hof”.



Plasma Cell (G&H, p. 59)

V. EMBRYONIC CONNECTIVE TISSUE

- A. Majority of connective tissue originates from **mesoderm**. Some originates from the neural crest (ectoderm).
1. Mesenchymal cells are present in developing organs and form a tissue type known as **mesenchyme**. These cells have a large nucleus and prominent nucleoli; the cytoplasm is barely visible. In between the cells is a large amount of ground substance with very few fibers. Mesenchyme is present only in the embryo and will give rise to all adult connective and support tissues.
 2. **Mucous Connective Tissue** – Has an abundance of ground substance with a larger number of collagen fibers and few cells (mostly fibroblasts). It is the principal component of the umbilical cord where it is referred to as **Wharton's jelly**.

VI. CONNECTIVE TISSUE PROPER

A. Loose (Areolar) Connective Tissue

1. Characterized by delicate, loosely arranged collagen with abundant ground substance and cells of all types. The most numerous are the fibroblasts and macrophages. All fiber types are present and it is well vascularized. This CT is designed for flexibility and is not very resistant to stress.
2. Distribution:
 - Sheaths around blood vessels and lymphatics
 - Serosal lining of the peritoneal and pleural cavities
 - Below epithelial surfaces such as the lamina propria of the GI and respiratory tracts, and areas of the dermis
 - Submucosa of the GI tract
 - Glands

B. Dense Irregular Connective Tissue

1. Collagen fibers predominate. The fibers are arranged in bundles oriented in **two or more directions** and without a definite structure. Compared with loose CT, this type of CT is less flexible, but much more resistant to stress.
2. Distribution:
 - Dermis of skin
 - Wall of vagina
 - Periosteum, perichondrium, epineurium
 - Capsules of organs

C. Dense regular connective tissue

1. Collagen fibers predominate, HOWEVER the collagen fibers are all arranged in **one direction**. This provides protection against prolonged stress exerted in one direction.

2. Distribution:
- Tendons (join muscle to bone)
 - Ligaments (join bone to bone)

CONNECTIVE TISSUE LAB (I) –Lots of slides today

SLIDE 17 – FETAL HAND

- This specimen shows mesenchyme with primitive Connective Tissue in different stages of differentiation. Cartilage and bone are forming in various areas. Look for cells below epithelium with large nuclei and wisps of ground substance. These are mesenchymal cells

SLIDE 18 – UMBILICAL CORD

- You are looking for Wharton's Jelly, so find the tissue with 3 vessels in it (hold on some white paper) and that will be the umbilical cord. The other stuff is placenta. The mucous connective tissue is the jelly, surrounding the vessels. You should see some fibroblast. Why is all the ground substance here?

SLIDE 3 – SMALL INTESTINE

- Look beneath the columnar epithelium that you previously examined. This area is the LAMINA PROPRIA. This is LOOSE CONNECTIVE TISSUE, even though it contains a large number of cells and fibers, so this is cellular loose CT.
- While here, look in the CT for:
- **Lymphocytes** – these appear as small dark nuclei with little or no surrounding pink cytoplasm.
- **Eosinophil** – Easy to see as their cytoplasm is bright orange and contains a blue nucleus with 2 lobes. RBC's do NOT contain a nucleus.
- **Fibroblasts** – Usually found in the looser, acellular CT below the lamina propria, called the submucosa. They look pale with wisps of collagen and other fibers attached and are elongated, cigar shaped in appearance.

SLIDE 19 – GALLBLADDER

- Look in the Lamina Propria (loose CT) and there will be, in addition to lymphocytes and fibroblasts, a number of **PLASMA CELLS** (derived from B-lymphocytes for future reference). They are plump, oval cells, with a basophilic cytoplasm, clock-face distribution of chromatin and eccentrically placed nucleus.

SLIDE 20 – LYMPH NODE

- Look for clumps of black particles. These indicate macrophages

SLIDE 48 – LUNG

- Look in the wide open spaces for large cells that have a light pink, frothy-looking cytoplasm. These are **ALVEOLAR MACROPHAGES** and enter the lung alveoli from the blood, ingest dirt and particles from the air, and are then coughed up and swallowed or expelled in the sputum (No spitting please).

SLIDE 1 – LIVER

- Look in the open spaces (sinusoids) between the hepatocytes. You will find large, light pink to brown cells, with a single nucleus. These are macrophages known as **KUPFFER CELLS**.

CONNECTIVE TISSUE LAB (b)

DENSE IRREGULAR CONNECTIVE TISSUE

SLIDE 9 – SCALP, SLIDE 10 – FOOT, SLIDE 22 – VAGINA

- Look under the epithelium. You will see bundles of eosinophilic collagen fibers that are closely packed, but randomly oriented (Therefore irregular). This is pretty much acellular loose connective tissue, unlike in the lamina propria

SLIDE 12 – TRACHEA

- Hold the slide against a white background and you will see a ring of tissue with some light blue **HYALINE CARTILAGE**. On the inside, luminal side, you previously looked at cilia. On the outside of the bluish hyaline cartilage, you will see quite dense layer of eosinophilic collagen fibers. This is the perichondrium and, despite the fact it looks to be laid down in the same direction, it is in fact **DENSE IRREGULAR CONNECTIVE TISSUE**. **This is an important concept in that you get to appreciate tissue type relationships and tissue locations.**

DENSE REGULAR CONNECTIVE TISSUE

SLIDE 23 – FINGER JOINT

- Here you should find a **TENDON**. This is very closely packed, parallel regular fibers inserting near the bony joint. The fibroblast nuclei are dark, long and wavy. You can also find an example of dense irregular CT in the periosteum on top of the bone.

CONNECTIVE TISSUE LAB (c)

SLIDE 24 – AORTA (H&E STAIN), SLIDE 25 – AORTA (VVG STAIN FOR ELASTIN), ELASTIC FIBERS

It is often difficult to see some fibers without a special stain. In slide 83 you can make out elastic fibers arranged into wavy elastic laminae. Take your lens slightly in and out of focus and these laminae will appear as refractile pink lines. Now look at slide 25 and see the very black VVG stained elastic fibers.

SLIDE 26 – EXTERNAL EAR, SLIDE 27 – EXTERNAL EAR (MONKEY)

Put slide 25 against a white background. The elastic cartilage is the dark ribbon down the center of the tissue. The elastic fibers appear as eosinophilic wisps in the matrix of the cartilage. In slide 26, the elastin has, once again, been stained with VVG and appears black.

SLIDE 28 – LIVER (VVG STAIN)

In this tissue you can see wispy **RETICULAR FIBERS**. Remember, reticular fibers can **not** be seen with H&E, are thinner than elastic fibers and are found in very cellular tissues (Liver, spleen, lymph nodes, etc)

BASEMENT MEMBRANE – SLIDE 12 – TRACHEA. Look under the columnar epithelium and there is a thick pink, eosinophilic line, composed of different layers and collagen (which ones)

DEMO slides of Muscle-tendon junction, Fetal pig foot and Intervertebral disc may be showing. Check front of lab