CHAPTER 4: CONNECTIVE TISSUE

Connective tissues are composed of three components: cells, extracellular fibers, and ground substance. Ground substance refers to the components of the extracellular matrix other than fibers, e.g., proteoglycans and glycoproteins. Classification of connective tissues is based on the types and relative abundance of the cells, fibers, and ground substance components that they contain.

Connective tissues are characterized by cells that tend to be widely separated from one another (in contrast to epithelial cells), and which are not specialized for either contraction (as in muscle) or conduction of action potentials (as in nerve).

Taken as a group, connective tissues serve many different functions. Some carry blood vessels and nerves to and from organs. They are an important site of gas and nutrient exchange with the blood since they directly surround most blood vessels. They are also a major site of inflammatory and immune reactions. They connect structures to one another (e.g. the tendons that connect muscle to bone), they fill the spaces between organs, they form the capsules of many organs, they provide the supporting framework (stroma) for almost all organs, they store energy in the form of adipose tissue, and they generate heat (brown fat). We will now consider the fiber types, the cell types, and the classification system for connective tissues.

CONNECTIVE TISSUE FIBERS

There are three major types of extracellular connective tissue fibers: collagen fibers, reticular fibers (actually a specialized type of collagen fibers), and elastic fibers.

COLLAGEN FIBERS:

This image shows the epithelium (epidermis) of the skin and the underlying connective tissue layer known as the dermis. Collagen is the most abundant protein in the body, and, like most proteins, it is eosinophilic in fixed tissues. It causes the dermis to stain pink with H&E. Although individual collagen fibers are not visible by light microscopy (LM), large bundles formed by many fibers (arrow) are easily seen.

This higher power view shows the two layers of the dermis. Above the arrow (but below the basement membrane of the epidermis) is the papillary layer. It is so named because it extends into the pale-staining projections called dermal papillae that interlock with downward projections of the epidermis. The papillary layer contains thin bundles of collagen fibers. In contrast the deeper layer of the dermis (known as the reticular layer) contains thicker, more easily visible fiber bundles (arrow).
In some cases it can be difficult to distinguish between closely packed collagen fibers and smooth muscles cells in H&E-stained sections, since both are eosinophilic and can be similarly shaped. Trichrome stains were developed to distinguish between the two by staining collagen and smooth muscle cells different colors. In the most commonly used trichromes (Mallory trichrome and Masson trichrome), collagen stains blue, as seen here. This section includes no smooth muscle, but it contains blue collagen fibers in the matrix of fibrocartilage that has been stained with Mallory trichrome. Compare this to H&E stained fibrocartilage in the next image.

In the matrix of fibrocartilage that has been stained with H&E the collagen fibers stain eosinophilic.

**ELASTIC FIBERS:**

Elastic fibers are able to stretch to ~150% of their length and then return to their original length without breaking. They are found mixed with collagen fibers in many connective tissues (e.g., the dermis), but are particularly abundant wherever their properties are needed to allow the tissue to flex or expand rapidly and then return to its normal shape. Examples include the walls of elastic arteries, the airways of the respiratory system, and the vocal ligament.

In the dermis, elastic fibers (arrow) are scattered among the more abundant collagen fiber bundles (yellow). Elastic fibers are usually not noticeable with H&E because they take up that stain very poorly. However, by using one of several different specialized stains it is possible to stain elastic fibers black, brown, or purple. Various counterstains can then be used to stain collagen a different color. Notice that elastic fibers tend to be much thinner than the collagen fiber bundles.

A mesentery is a thin sheet of connective tissue that is covered on both surfaces by a simple squamous epithelium (mesothelium). Mesenteries connect intraperitoneal organs to the body wall, and carry the blood vessels, nerves, and lymphatics that supply these organs. In this image an intact (unsectioned) mesentery has been spread flat on the slide, stained for elastic fibers (arrow), and counterstained with H&E to reveal collagen fiber bundles (lighter pink). The varied
orientation of the elastic fibers allows the mesentery to stretch in many different directions. Elastic fibers that are under tension are stretched (straightened), while those that are relaxed are coiled (arrow).

In the walls of muscular and elastic arteries, elastic fibers may be so abundant that they fuse to form fenestrated sheets (laminae) of elastic tissue. The arrow lies in the lumen of a muscular artery and indicates the internal elastic lamina (internal elastic membrane) of the vessel. Further out from the lumen additional elastic tissue is present in the form of thinner fragmented sheets and individual fibers.

Many fenestrated elastic laminae are found in the muscular layer (tunica media) of elastic arteries such as the aorta. The tunica media lies just outside the tunica intima indicated by the arrow.

In the muscular layer of elastic arteries the elastic laminae are so thick that they may even be visible after H&E staining, i.e., without a specialized elastic tissue stain. They alternate with layers of smooth muscle cells. This is one of the few places in the body where specialized stains may not be necessary in order to demonstrate elastic tissue.

**RETICULAR FIBERS**

Reticular fibers are the major type of fiber present as the supportive element in soft organs such as liver, spleen, lymph nodes, and bone marrow. They are actually composed of a type of collagen (collagen type III). Special techniques such as silver stains or the PAS procedure can be used to stain reticular fibers (arrow) and distinguish them from other types of collagen. Silver techniques stain reticular fibers black. Reticular fibers are not limited to soft organs. They are also present along with collagen and elastic fibers in structures such as blood vessels.

Reticular fibers commonly form a delicate network (or reticulum) that provides support while also allowing motile cells (such as the lymphocytes in this lymph node) to move about within the organ.
CONNECTIVE TISSUE CELLS

Connective tissue cells are often divided into fixed (or resident) cells vs. wandering (transient or immigrant) cells. Resident cells are those that either arise in situ in the connective tissue or migrate into it, and are present there for long periods of time. They tend to be somewhat less motile than wandering cells and their numbers tend to fluctuate less over time. Resident cells include fibroblasts, mast cells, adipocytes, and some macrophages. Wandering cells are derived from blood leukocytes that migrate into the connective tissues in response to specific stimuli such as inflammation or infection. Their numbers spike temporarily in the connective tissues and then decline when the condition is resolved. Some wandering cells, such as lymphocytes, are able to return to the blood and recirculate, but other types normally die in the connective tissues after carrying out their functions there.

Macrophages are unusual in that they can be counted among both the resident and the wandering cell populations. There is a resident population in most connective tissues, but their number can increase greatly in circumstances such as inflammation or infection when large numbers of monocytes migrate into the connective tissues and differentiate into macrophages.

RESIDENT CELLS

Fibroblasts are the major type of resident cell in most connective tissues. They arise from stem cells in the connective tissue and remain there throughout their lifetime. In almost all connective tissues fibroblasts are the cells that produce the extracellular fibers and the components of ground substance. One exception is in the muscle layer of blood vessel walls, where smooth muscle cells carry out these functions.

Fibroblasts are elongated cells with flattened oval nuclei. Their tapering cytoplasm is often difficult to see, especially in a dense connective tissue where the abundant collagen fibers stain the same eosinophilic color as fibroblast cytoplasm.

This higher power view illustrates the extremely attenuated shape of a fibroblast. Cytoplasm is just barely visible at either end of the elongated nucleus. The cell is surrounded by many collagen fiber bundles.

Macrophages (arrow) are one of the two major types of phagocytic cells in humans. They are lighter staining than most cells, and often have an irregular nucleus that may be lumpy,
folded, horseshoe-shaped, etc. Due to intense phagocytic activity, the plasma membrane is often indistinct or ragged in appearance. To conclusively identify a macrophage, it is often necessary to see some phagocytized material within the cytoplasm, or large, empty-looking vacuoles that are probably also formed via phagocytosis. Both are present in this cell.

Mast cells regulate the permeability of small blood vessels, especially postcapillary venules, by releasing histamine (stored in secretory granules) and leukotrienes (newly synthesized) that enhance vascular permeability. These round or oval cells have a round or oval nucleus and a considerable amount of cytoplasm, which is normally packed with large secretory granules. These granules may stain metachromatically with certain dyes, meaning that they take on a color that is different from the color of the stain. Because of their function, mast cells are often found near small blood vessels.

White adipocytes (white fat cells) are often scattered in small groups in loose connective tissues, but they are the major cell type present in white adipose tissue. When mature, they contain a large lipid droplet that fills most of the cytoplasm and compresses the nucleus at the side of the cell. This gives the cell a “signet ring” appearance where the nucleus resembles the single large stone of the ring. The lipids are usually extracted by the organic solvents used in tissue preparation, so that most of the cytoplasm appears empty.

Lymphocytes are one of the five types of human white blood cells (leukocytes). They are found in many connective tissues, especially in regions that are likely to encounter foreign antigens, such as the connective tissue layer (lamina propria) that lies just beneath the epithelium of the small intestine. Lymphocytes are the major effector cells in immune responses.

When not active in an immune response, lymphocytes are small cells with a round or slightly indented (kidney-bean shaped) nucleus that is extremely heterochromatic. The arrow indicates
a group of lymphocytes passing through a lymph node. They have such a small amount of cytoplasm that it is often difficult to see it except at very high magnification. When activated, lymphocytes increase in size, their nucleus becomes more euchromatic, the relative amount of cytoplasm increases, and the cells divide.

When the number of lymphocytes in any given region increases significantly, the region appears very basophilic (arrow). This is due to the presence of many closely packed dark-staining lymphocytes. Lymphocytic infiltration indicates that there is an ongoing immune response at that site.

During an immune response some B lymphocytes differentiate into plasma cells, which synthesize and secrete antibodies in response to foreign antigen. Plasma cells have an eccentric nucleus (i.e., pushed to the side of the cell as opposed to being centrally located). The nucleus has clumps of heterochromatin distributed around its edge, often with one central clump. This pattern is called either clockface (because the clumps look like the numerals on the face of an analog clock) or cartwheel (because the areas of euchromatin look like the spokes on the wheel of a cart). The cytoplasm is strongly basophilic due to the RER where the antibodies are produced. There is often a light-staining patch of cytoplasm near the nucleus, which contains the well-developed Golgi, where antibodies are packaged into small secretory vesicles.

Eosinophils (arrow), basophils, and neutrophils are the three types of human granulocytes (a class of white blood cells). Granulocytes leave the blood and enter the connective tissues to carry out their functions. Eosinophil numbers increase in connective tissues during parasitic infestations and in response to allergic reactions.

This image shows an eosinophil in a blood smear rather than in connective tissue. Eosinophils have a lobed nucleus (usually bi-lobed, but occasionally tri-lobed) and numerous cytoplasmic secretory granules that are very large and very eosinophilic (hence the name eosinophil). Eosinophils are actually more common in connective tissues than in blood.
Basophils, seen here in a blood smear, have a lobed nucleus that is usually obscured by numerous large and highly basophilic secretory granules (hence the name basophil). The basophil nucleus is the least lobulated of all the granulocytes. Basophils are very similar in function to mast cells in that they increase the permeability of postcapillary venules by releasing mediators such as histamine and leukotrienes. Over-activation of basophils and mast cells leads to allergic reactions such as hay fever, hives, and asthma.

A mature neutrophil, seen here in blood, has a nucleus with 3-5 lobes (usually 3) that are connected by very thin strands. They have small cytoplasmic secretory granules that do not stain well (hence the name neutrophil – neither eosinophilic nor basophilic). Neutrophils, like macrophages, are highly phagocytic. They are especially adept at phagocytizing bacteria.

CLASSIFICATION OF CONNECTIVE TISSUES

Although it is considered less important than in the past, connective tissue classification is still included in most histology textbooks. Mature connective tissues are usually divided first into connective tissue proper vs. specialized connective tissues. Specialized connective tissues include white adipose tissue, brown adipose tissue, cartilage, bone, reticular connective tissue, blood, and hematopoietic tissue (bone marrow). With the exception of adipose and reticular tissues, which are described below, the other specialized forms will be considered in later units. Connective tissue proper is considered to be the common or “ordinary” connective tissue of the body. Embryonic connective tissue is often considered a third type that is separate from the adult forms.

Classification of connective tissue proper is based on criteria such as the relative abundance of fibers vs. ground substance in the matrix (loose vs. dense), and the arrangement of the fibers (regular vs. irregular). The categories usually described are dense irregular connective tissue; dense regular connective tissue; and loose connective tissue (or areolar connective tissue), which includes the subtype known as loose cellular connective tissue.
A mesentery is an example of a loose (or areolar) connective tissue. It is characterized by large amounts of ground substance and relatively few fibers in the matrix. The number of cells per unit area is greater than in a dense connective tissue. Fibroblasts usually predominate, although the variety of cell types is greater than in dense connective tissues.

The lamina propria of many organs including small intestine is an example of a subtype of loose connective tissue that is referred to as loose cellular connective tissue. The matrix characteristics are similar to those of ordinary loose connective tissue (few fibers and abundant ground substance) but many more cells are present than in ordinary loose connective tissue. It may in fact be difficult to see fibers because of the abundance of cells. In the lamina propria most of these additional cells are leukocytes, especially lymphocytes and to a lesser extent plasma cells.

In this image of the small intestine, the loose cellular connective tissue layer called the lamina propria lies just above a thin layer of smooth muscle indicated by the arrow. Most of the cells in the lamina propria are lymphocytes or plasma cells. Several dark-staining intestinal glands are also present in this layer. In a loose cellular connective tissue it may be difficult to see extracellular fibers in the matrix because of the extreme abundance of cells, making it difficult to appreciate that this is in fact connective tissue. Notice that the connective tissue layer (called the submucosa) that lies below the smooth muscle is much less cellular, and that abundant eosinophilic collagen fiber bundles are obvious there.

A dense connective tissue contains relatively more fibers and less ground substance in its matrix than does a loose connective tissue. There tends to be a more restricted variety of cell types, with the majority of cells being fibroblasts. The deeper layer of the dermis of the skin (the reticular layer), which is indicated by the arrow, is an example of a dense connective tissue.
Dense connective tissues are subdivided into dense regular or dense irregular. The reticular layer of the dermis is an example of a dense irregular connective tissue. In dense irregular connective tissue the fiber bundles are oriented in many different directions so that it would be impossible to look at one bundle and predict the direction in which its neighbors would be oriented. Note how few cells are present, and that most of them have the elongated nuclear shape characteristic of fibroblasts.

In a dense regular connective tissue the fibers are oriented in a predictable direction. In most cases, as in this longitudinal section (ls) of a tendon, they will all be oriented in the same direction. In a few cases, such as the cornea of the eye, the fibers are arranged in layers, with all the fibers in a given layer having the same orientation but adjacent layers being oriented in different directions, like the layers in a piece of plywood. Note that, as in this relaxed tendon, the fiber bundles in a dense regular connective do not have to be perfectly straight. They only have to be oriented in a predictable pattern.

A ligament is another example of a dense regular connective tissue. It differs from a tendon chiefly in its location. A tendon connects muscle to bone while a true ligament connects bone to bone. Histologically they are very similar and you would not be expected to distinguish between them at high magnification. Recall that in a trichrome-stained section collagen will be stained some distinctive color, in this case blue.

The top half of this image (indicated by the bar) shows the dense regular connective tissue of a tendon. The bottom half shows skeletal muscle cells cut in cross section (with the nuclei of these multinucleated cells at the periphery of each cell). You can see how useful a trichrome stain would be in cases such as this to distinguish between the two tissue types.
Reticular tissue is a meshwork formed by reticular fibers and the reticular connective tissue cells that make them. Reticular connective tissue cells (or reticular cells) are closely related to fibroblasts but tend to cling to and surround the reticular fibers, whereas fibroblasts lie between the fibers that they make but do not encircle them with cytoplasmic processes. Reticular tissue makes up the stroma of soft tissues and organs such as adipose tissue, liver, bone marrow, lymph nodes, and spleen. In this section the reticular fibers have been stained with a reticular stain (a silver stain to be more precise).

Beneath the dermis of the skin lies a layer called the hypodermis, which is composed mainly of adipose tissue. Adipose tissue is simply a loose connective tissue in which adipocytes (usually white adipocytes) predominate. They often form lobules of fat separated from each other by sheets (septa) of denser connective tissue. The arrow lies within one such lobule, composed of many individual adipocytes. The reticular layer of the dermis (dense irregular connective tissue) lies at the top of the image. An eccrine sweat gland is embedded in the fat lobule, and the duct of a sweat gland is seen coiling upward through the dermis.

This is a high magnification image showing several adipocytes within a lobule of white fat. Recall that each mature white adipocyte has a single major lipid droplet occupying the center of the cell and pushing the nucleus and the rest of the cytoplasm to the periphery. A silver stain of this section would have revealed delicate reticular fibers between adipocytes.

**BROWN FAT**

Brown fat is rare in humans, being most common in newborns. No images of it are available in this laserdisk collection. Brown fat cells (brown adipocytes) are multilocular (having many small lipid droplets in each cell) rather than unilocular (having one major lipid droplet) as are white adipocytes. The nucleus of a brown fat cell tends to be centrally located rather than peripheral. One added complication is that during the differentiation of white adipocytes the cells initially have many small droplets that gradually merge to form one large droplet. Therefore immature white adipocytes could be mistaken for brown fat. We will never ask you to distinguish between brown fat and immature white fat.
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<th>Frame</th>
<th>Stain/Method</th>
<th>Magnification</th>
<th>Question</th>
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<td>H&amp;E</td>
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<td>1. Does the arrow indicate collagen fibers, elastic fibers or reticular fibers?</td>
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<td>400x</td>
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<td>7050</td>
<td>H&amp;E</td>
<td>160x</td>
<td>3. This section has been stained with something other than a silver stain or PAS. Does the arrow indicate collagen fibers, elastic fibers or reticular fibers?</td>
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<td>H&amp;E</td>
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<td>6657</td>
<td>Silver stain</td>
<td>80x</td>
<td>7. Identify this type of connective tissue fiber.</td>
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<td>Wright’s Stain</td>
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<td>10. Classify this type of connective tissue.</td>
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<td>19. Classify this type of connective tissue.</td>
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ANSWERS TO QUIZ #3: CONNECTIVE TISSUE
NOTE: Statements in brackets provide additional information, but that information is not required in order for the answer to be considered correct.

1. Collagen fibers
   Frame  6428

2. Macrophage
   Frame 6037

3. Elastic fiber
   Frame 7050

4. Basophil
   Frame 12551

5. Eosinophil  [Ideally the large cytoplasmic granules would be somewhat redder to help distinguish the cell from a basophil. The fact that the granules do not obscure the shape of the nucleus makes it more likely that the cell is an eosinophil.]
   Frame 12473

6. Plasma cell
   Frame 6153

7. Reticular fiber
   Frame 6657

8. Neutrophil
   Frame 12311

9. Mast cell  [There are two mast cells in this image.]
   Frame 6124

10. Dense irregular connective tissue
    Frame 7206

11. Reticular connective tissue
    Frame 6663

12. Loose connective tissue
    Frame 7161
13. **Fibroblast**  
   Frame 6016

14. **White adipose tissue** [Usually just referred to as adipose tissue since brown adipose tissue is so rare.]  
   Frame 7420

15. **Mast cell** [Compare this one with the mast cell in Frame 6124 to appreciate that some variation in appearance between tissue specimens is likely for all cell types. The most helpful characteristics for a mast cell are the numerous large cytoplasmic granules and the fact that the nucleus is not lobed (which helps to distinguish mast cells from the granulocytes that have large granules, namely eosinophils and basophils.]  
   Frame 6088

16. **Elastic fibers** [The layers of fibers separated by other tissue in between strongly suggests that this is the wall of an elastic artery, which is one of the common locations for abundant elastic fibers. In other tissues they are not so regularly arranged.]  
   Frame 17351

17. **Dense irregular connective tissue** [The tissue on the right of the micrograph has thinner fibers and relatively more cells, and so could be classified as a loose connective tissue.]  
   Frame 7212

18. **Dense regular connective tissue**  
   Frame 7318

19. **Loose cellular connective tissue**  
   Frame 7071