This lecture will present the histology of connective tissue proper, the tissue that fills in a continuous compartment bounded by epithelium and the wrappings of muscle providing a pathway for blood vessels, lymphatic vessels and nerves through muscle and organs, and, that connects muscle to bone and bone to bone. Connective tissues are composed of cells, fibers, and a ground substance that fills between the cells and fibers. The dominance of one of these three components forms unique connective tissue types. Illustrated here are upper left, mesenchyme (embryonic) connective tissue (ground substance is dominant), lower left, loose connective tissue (cells and ground substance dominant), upper right, dense irregular connective (non-parallel fibers are dominant), and lower left, dense regular connective tissue (parallel fibers are dominant).

These are the relevant resources that relate to the content of this lecture. The lecture PDF downloadable from the Course Website contains all of the slides and the narrative of this lecture. The laboratory exercise is found in the WebMic Study Guide and is used along with the online program, WebMic.

These are the vocabulary of terms related to connective tissue proper. Look for these terms as you view this lecture. One way of enhancing your learning is to perform a Google search on the Internet for each of these terms. Learning is best facilitated by multiple modes of input.
Learning Outcomes

- After completing a study of this connective tissue lecture and working through the related laboratory exercise, you should be able to:
  - define connective tissue proper vocabulary terms.
  - state the 3 basic ingredients in connective tissue.
  - recognize embryonic (mesenchymal) connective tissue.
  - understand the origin of connective tissue.
  - state the subtypes of connective tissue proper.
  - name and identify the fixed & wandering cells of connective tissue.
  - state the function of the fixed & wandering cells of connective tissue.
  - name the types of fibers in connective tissue and give their function.
  - state the components of ground substance and its function.

These are the learning outcomes of this lecture. At the end of the lecture you will be presented with a few questions to aid in assessing your understanding and retention of the important concepts in this lecture.

Lecture Topics

- Connective Tissue Concepts
- Types of Connective Tissue
- Connective Tissue Proper Classification Scheme
- Fibers of connective tissue
- Ground Substance of connective tissue
- Cells of connective tissue

Each of the topics listed on this slide is hyperlinked to the first slide that begins that topic. You can jump to any topic. When you reach the first slide for a certain topic, you will find on that slide a button to click that will return to this slide. This should make it convenient when it comes time when reviewing if you do not need to view and listen to the entire lecture.

Connective Tissue Concepts

- Classification of connective tissue
  - Based on composition & organization of the cellular & extracellular components and on special functions
  - To classify connective tissue 3 factors must be considered
    - The kinds of cells present
    - The types of fibers present-extracellular component
    - The character of the ground substance-extracellular component

All connective tissues are composed of cells, fibers and ground substance. Fibers and ground substance are extracellular. Compared to epithelial tissue, the extracellular component takes on much more significance in the histological architecture. Therefore, classifying connective tissue involves the identification of the type of cells, fibers and ground substance present.
There are three types of connective tissue: embryonic, connective tissue proper and specialized connective tissue. This lecture will briefly present the histology of embryonic tissue to contrast it with the adult connective tissue proper. The focus of this lecture is on connective tissue proper. Separate lectures will be presented on the specialized connective tissues.

Connective tissue proper is composed of cells that are either fixed or mobile (wandering), and an extracellular matrix composed of fibers and ground substance. The proportion of cells, fibers and ground substance varies with the types of connective tissue proper. Cells are dominant in loose connective tissue, fibers are dominant in dense connective tissue and ground substance is dominant in specialized connective tissues – cartilage, bone and mesenchyme (embryonic). The term Matrix refers to all of the extracellular components. The fixed cells are the stable ones always found in connective tissue. If adipocytes (fat cells) are dominant then it becomes adipose tissue, not connective tissue proper. Adipose tissue is classified as one of the specialized connective tissues. Fixed cells are cells that are always present in connective tissue proper, especially all of them in loose connective tissue. The mobile cells are ones that move into and out of connective tissue and their numbers are influenced by inflammation in response to acute or chronic infection.

Embryonic mesenchyme connective tissue is composed of cells (in this case called mesenchymal cells), fibers and ground substance. This specimen of mesenchyme was prepared routinely and stained with hematoxylin and eosin. The spaces where the ground substance is normally located but since the tissue preparation used water, the ground substance was washed out so there was nothing to stain. Mesenchyme is the tissue that has the potential to differentiate into all kinds of adult connective tissues. The next slide will illustrate the many cell types that differentiate from mesenchymal cells.
Histology of Connective Tissue Proper

The mesenchymal cell gives rise to many different adult cell types that we will encounter as the course proceeds. For this lecture, we emphasize the fact that mesenchymal cells differentiate into fibroblasts, the cells of connective tissue proper. Fibroblasts synthesize and secrete the fibers and ground substance of connective tissue proper.

Loose Connective Tissue, illustrated by the left drawing and image, is characterized by widely separated cells, medium to fine fibers and a significant presence of ground substance as in mesentery and the delicate tissue underlying most of the epithelium of the gastrointestinal tract. Loose connective tissue functions to provide easy access of immune cells, blood vessels and nerves to epithelia. It also serves as an intermediate tissue between skeletal muscle fascicles and blood vessels and nerves traveling through muscle. Dense Irregular Connective Tissue is characterized by a dominant presence of thick collagen fibers arranged in different directions and planes as in the deeper reticular layer of the dermis of skin. This tissue functions to resist tension in several directions as occurs in skin and in the containment and wrapping of some organs. Dense Regular Connective Tissue is characterized by the dominant presence of thick collagen fibers arranged parallel to one another as in tendons and ligaments. Collagen fibers are very strong. When they are arranged in parallel and anchored, for example, into bone and muscle, they efficiently conduct force generated in muscle to bone. When they are connected from bone to bone, they serve to hold two bones in a normal anatomical position.
Loose connective tissue occupies a continuous compartment bordered by the basal laminae/basement membranes of various epithelia and the basal laminae of muscle cells and supporting cells of nerves. Where loose connective tissue underlies epithelium, it is called lamina propria and where it surrounds epithelial cells as in a gland, it is called the stroma (the supporting framework of an organ). Where it underlies epithelia, it functions as a transporting medium between blood vessels and the epithelium. Where it surrounds muscle and nerves, it serves to define and confine the muscle and nerve serving as ‘connective tissue wrappings’.

In this continuous compartment the cells of connective tissue produce products that function to provide strength and support. The cells make most of the composition of basement membranes. In loose connective tissue, there is water but it is not sloshing around because it is bound to large molecules like hyaluronic acid. The long fibers provide strength and elasticity and other molecules like glycoproteins hold fibers in place and fix cells to fibers so the cells are not just floating around.

There are three types of fibers that contribute to the matrix of connective tissues. Collagen Fibers that are either very narrow or fine and form a network in loose connective tissue or robust/wide occurring in bundles in dense connective tissue. Reticular Fibers are made up of a special variant of collagen, they are very thin (about 1 micron) and form a supporting framework for the cells of glands and organs like salivary glands, liver, pancreas, kidney etc. Elastic Fibers are thin, straight and branch. They occur in single strands and form a network in the dermis of skin and in blood vessels. This provides elasticity, which allows the skin to be stretched in order to conform to growth of muscles and bones. In blood vessels, this elasticity allows for adjustments in changes in blood pressure.
A collagen fiber is assembled outside of the fibroblast that secretes the precursor to tropocollagen, a molecule called procollagen. An extracellular enzyme secreted also by the fibroblast, pro-collagen peptidase cleaves the uncoiled ends of the procollagen molecule thus producing tropocollagen or otherwise known as the collagen molecule. The triple helix of two alpha 1 and one alpha 2 peptide chains is shown in color. Then collagen molecules are assembled into collagen microfibrils by the cross-linking of covalent bonds formed between lysine and hydroxylysine molecules in the adjacent molecules of collagen. These microfibrils are banded as can be seen in the transmission electron micrograph (TEM) (lower left). Groups of fibrils are then assembled to form larger diameter structures known as collagen fibrils. Finally through the aggregation of fibrils a collagen fiber is formed. Collagen is a very strong molecule capable of great tensile strength as you can imagine when you see a world class weight lifter lift a weight several times the lifter’s body weight. Tendons are made up almost 95% collagen. The next slide illustrates what can happen if collagen is defective lacking its normal tensile strength.

Ehlers-Danlos syndrome demonstrates the important role played by the tensile strength of the collagen molecules in collagen fibers. This syndrome is the result of an inherited defect in procollagen peptidase and a mutation of the gene encoding the enzyme lysyl hydroxylase. Both of these enzymes are of critical importance in the synthesis and formation of normal collagen. As a result, the tensile strength of collagen is greatly reduced. Defects in collagen in this syndrome result in hyperelasticity and abnormal range of motion of joints. The hyperelasticity of the skin in this pathological condition illustrated here not only demonstrates the loss of restraint from stretching of the skin due to the collagen defect, but, at the same time, shows how much the skin can be stretched which is due to elastic fibers in the skin. The hyperextension of the joint shown here that is typical of patients with Ehlers-Danlos syndrome demonstrates how important collagen is in the normal range of motion of the joints. The next slide illustrates elastic fiber composition.
The light micrograph graphically illustrates the strong acidophilic staining of elastic fibers. Note that elastic fibers, unlike collagen fibers have both an amorphous component made up of elastin and a filamentous component made up of fibrillin. Note the close relationship between the fibroblasts and the forming elastic fibers. Elastic fibers are ubiquitous in the body. They are throughout the dermis of the skin, in the wall of the trachea, and all of the airways of the lung, in the aorta and the large arteries and many other sites.

Reticular fibers are very thin, on the order of 1 micron in diameter compared to collagen fibers that are 10 microns or more in diameter. Reticular fibers have a rich coat of carbohydrate on their surface that reacts with silver salt solution resulting in silver deposited on the fiber. A silver stain was applied to this specimen. Reticular fibers are, therefore, argyrophilic (they have an affinity for silver in solution). Reticular fibers provide a supporting framework for the cellular constituents of various tissues and organs. This is a specimen of a lymph node. Note how the lymphocytes are cradled in a reticular fiber network.

In between cells and fibers in connective tissue proper, we find ground substance. Ground substance consists of glycosaminoglycans (GAGs), proteoglycans, and multiahesive glycoproteins. Proteoglycans are extracellular protein complexes of glycosaminoglycans. Glycosaminoglycan molecules consist of several hundred carbohydrate molecules linked. Glycosaminoglycans such as keratan sulfate or chondroitin sulfate are attached in repetitive fashion to a core protein to form a molecule of proteoglycans. You can think of this molecule as shaped like a test tube cleaning brush. In loose and dense connective tissue proteoglycans are present in variable amounts. Also, a molecule known as hyaluronan (hyaluronic acid) is present in loose and dense connective tissue, more in loose than in dense. Hyaluronan is a non-sulphated glycosaminoglycan made up of approximately 2,500 repeating disaccharide units of N-acetylglucosamine and D-glucuronic acid much longer than glycosaminoglycans. It is present free in most
connective tissues. It is a very large molecule and can entrap a large volume of water that contributes to the turgidity of connective tissue. Proteoglycan aggregates consist of proteoglycans molecules attached to a backbone of hyaluronan by linker proteins. Proteoglycan aggregates make up the larger part of the ground substance of cartilage. More about proteoglycan aggregates is in the presentation on cartilage.

This slide compares Hyaluronic Acid (hyaluronan) with other large molecules - globular protein, glycogen, spectrin (intermediate filament protein in erythrocytes) and collagen. Note the enormous size of the Hyaluronic Acid molecule. This is the molecule that fills its spaces with water when we are hydrated and loses it when we are dehydrated. Also, its capacity to hold water seems to diminish with aging. Hyaluronic acid depletion in the skin of aging humans has sparked a thriving industry although its benefits are questionable. Infection by two common bacteria, staphylococcus and streptococcus, produce dramatically different results when they invade the dermis of the skin where hyaluronic acid is prevalent. Hyaluronidase is an enzyme that will degrade (breakdown) the molecules of hyaluronic acid. Staphylococcus bacteria do not secrete hyaluronidase and therefore staph infections tend to produce infections that are contained within a restricted area, like boils. Streptococcus bacteria on the other hand, do secrete hyaluronidase. Strep infections tend to spread. More about the infection of skin with these bacteria will be presented in the lecture on the histology of skin.
The text on this slide argues for the benefit of hyaluronic acid as an anti-aging chemical. The text was extracted from a longer article that can be accessed by clicking the link at the bottom of the slide. If you look in any pharmacy you will see a variety of ‘over the counter’ products that contain hyaluronic acid that claim to restore worn out cartilage and make joints function more smoothly.

Now, the fixed and mobile cells of connective tissue proper will be described and illustrated. The fibroblast synthesizes several types of collagen (see list lower right), elastic fibers and the molecules of the ground substance. Fibroblasts, when actively synthesizing these molecules have a bluish stained cytoplasm with hematoxylin (basophilic due to the rough endoplasmic reticulum) and a prominent nucleolus in a euchromatic nucleus (nucleolus is the source of ribosomes and the euchromatin is the distended form of DNA in which transcription is taking place). The collagen fibers are secreted as tropocollagen and then, the tropocollagen through enzymatic activity are assembled outside of the fibroblast into collagen fibers. If fibroblasts are not synthesizing and secreting fibers and ground substance they are said to be inactive and usually are called fibrocytes. They are thin, flat with less basophilic cytoplasm and a more heterochromatic nucleus. Fibroblasts are present in all types of connective tissue proper.
Tissue macrophages, fixed cells, come from monocytes that emerge from the blood. Once outside of the blood, a monocyte will either die or be transformed into a cell that has great ability to take foreign particles, bacteria, viruses and other foreign substances. Macrophages can ingest whole cells or fragments of cells that may be the remains of an intense inflammatory reaction to a bacterial infection. Macrophages tend to accumulate in chronic infections. The process whereby the macrophage takes in substances is called phagocytosis. It contains what it has phagocytosed in vacuoles and these eventually fuse with lysosomes at which point the substance is digested. Note in the drawing the multiple vacuoles. In the LM (lower left), a macrophage is shown that has taken in inert matter such as carbon particles. Note the electron dense rounded structures in the TEM of the macrophage. These are lysosomes containing acid phosphatases and other enzymes.

Adipocytes (fat cells), another fixed or permanent cell in connective tissue, contain large vacuoles of lipid. They may be found in small numbers in connective tissue proper. In large numbers, they form adipose tissue. Adipose tissue will be presented in a different lecture. Mast cells are cells that contain many basophilic granules, are oval shaped and have an ovoid nucleus located in the middle of the cell. Mast cells are distributed throughout the body’s connective tissues intimately associated with small blood vessels. The substances that mast cells secrete are involved in inflammation. For instance, histamine increases the permeability of small post capillary venules so that inflammatory cells can more readily escape from the vessel to migrate to the site of infection.

The monocyte circulates in the blood. Part of its life cycle and function is to move out of the vessels into connective tissue and in this manner, it is a wandering or mobile cell of connective tissue. However, the transformation into a macrophage results in a family of macrophages. Macrophages derived from Monocytes may aggregate, in the case of a chronic infection, to form giant cells that surround and sequester foreign material, or they may transform into a family of cells that have phagocytic capability. The generic macrophage of loose connective tissue participates in acute and chronic infections. The specialized macrophages listed here that are found in bone, liver, spleen, bone marrow, lung and the central nervous system each have specific phagocytic functions in those organs. The unique macrophages of each organ will be further explained during the lectures on those organs.
Lymphocytes and plasma cells are mobile cells in connective tissue proper. Lymphocytes circulate in the blood. One type of lymphocyte, the B Cell, when it emerges from the blood transforms into an antibody secreting cell, the plasma cell. Note the transformation from a round cell with very little cytoplasm to a cell that has much more cytoplasm that is basophilic reflecting the large amount of rough endoplasmic reticulum needed to synthesis the immunoglobulin (antibody).

Eosinophils and neutrophils are additional circulating blood cells that emerge under certain conditions from the blood and enter connective tissue. Eosinphils function to sequester and destroy immune (antigen-antibody) complexes and neutrophils inactivate and sequester bacteria. These cells will be presented and explained in greater detail in the lecture on peripheral blood histology.