THE CONNECTIVE TISSUES

The connective tissues generally consist of an indigenous population of cells surrounded by fibers and an amorphous ground substance. These tissues connect and anchor structures and give support to the body and its organs. The proportion of cells to fibers and ground substance varies greatly from one type of connective tissue to another.

ORIGIN OF CONNECTIVE TISSUES

Connective tissues are believed to arise from an embryonic tissue called mesenchyme. Mesenchyme arises from mesoderm, an embryonic germ layer, and is composed of stellate or fusiform cells embedded in an extensive, jelly-like ground substance. Mesenchyme is the stem tissue of all the connective tissues of the body. Connective tissues fall into four main categories:

1. Loose connective tissues (adipose tissue)
2. Dense connective tissues (tendon)
3. Support tissue (cartilage and bone)
4. Blood and blood forming tissue (red marrow)
1. Mesenchymal cells - embryonic cells which persist in the adult and are capable of differentiation and proliferation during regeneration.

2. Fibroblasts - large, flat, branching cells which appear spindle-shaped in a side view. In mature tissue they are quiescent. After injury, they begin to form new fibers. Under the electron microscope, the activated fibroblast shows the extensive rough ER needed for the synthesis of collagen and elastin fibers. Proteoglycans, a chief component of the ground substances of connective tissues are also synthesized by these cells. Fibroblasts have large, oval and faint staining nuclei with one or two conspicuous nucleoli.

3. Macrophage (histiocyte) - next in abundance to the fibroblasts in loose connective tissue, these cells are initially non-motile. During inflammation, they become very actively amoeboid and phagocytic (“angry macrophages”). They readily engulf blood cells, bacteria, dead cells and debris digesting this material with powerful enzymes. The nucleus of this cell type is often indented and dark staining. Nucleoli are not conspicuous. These cells are an important component of the reticuloendothelial system (RES) located in the spleen, liver, lymph nodes and other organs.

4. Adipose cells - These cells are commonly seen in loose connective tissue (areolar). They are often found arranged around small blood vessels. Initially they resemble fibroblasts but with
numerous vacuoles of fat droplets. In adult cells, the droplets coalesce bloating the fat cells with a huge fat vacuole.

5. Leukocytes - are white blood cells which wander into the connective tissues surrounding blood vessels. Eosinophiles are very common throughout the respiratory and digestive tracts, as well as in active mammary tissue. Neutrophiles are found at sites of inflammation. Plasma cells, derived from B lymphocytes, are common in areas of chronic inflammation.

6. Mast cells - are large cells (20 to 30 um) filled with deeply basophilic granules which often obscure the nucleus. They probably arise from mesenchyme cells in the body tissues and are usually adjacent to blood vessels. Like the blood basophils, which they closely resemble, these cells contain mediators of immediate hypersensitivity such as histamine, heparin and serotonin.

7. Melanocytes - are pigment cells found in the connective tissues of the skin and choroid coat of the eye. The melanin produced by these cells is known to absorb ultra violet light.
CONNECTIVE TISSUE FIBERS

A. **Collagen fibre**- a protein forming extracellular fibers in practically every tissue of the body. The amount of collagen in a particular connective tissue depends on the density of the tissue. In loose connective tissue collagen is sparse. In dense connective tissue collagen is abundant. The arrangement of collagen fibers will vary with the function of that tissue in different regions of the body, for example:

1. In tendon and ligament, fibers are thick, long and grouped in parallel assembly for tensile strength.

2. Fibers are helically arranged around the long axes of tubular structures, i.e., blood vessels, intestine and glandular ducts.

3. In cartilage and bone, calcium phosphate salts deposit on collagen fibers determining the orientation of these structures.

4. In the cornea of the eye, layering of collagen fibers at angles to one another, as well as, the spaces between layers determine the refractive ability of the cornea.

**Structure of Collagen**

Each collagen fiber consists of aggregates of tropocollagen molecules. Each tropocollagen molecule is composed of 3 polypeptide chains which are helically arranged around each other and cross-linked for structural strength. The staggered arrangement of tropocollagen molecules in a collagen fiber produces a repeating cross banding on the fiber which can be seen under EM.
Cells Producing Collagen

Collagen is generally synthesized by cell types derived from mesenchyme, e.g., fibroblasts, osteoblasts, chondroblasts and smooth muscle cells. The collagen of the basal lamina of the basement membrane is synthesized by epithelial cells of various types.
B. **Elastic Fibers** - are protein in nature and capable of returning to their original length after being stretched. The molecules which compose these fibers are synthesized in fibroblasts and smooth muscle cells. They are not found in bundles but occur as solitary fibers. Tissues and organs subject to expansive forces contain elastic fibers, such as, the walls of arteries, the vocal cords, skin, the trachea and the bronchi.

**Figure 4.8f  Connective tissues.**

<table>
<thead>
<tr>
<th>(f) Connective tissue proper: dense connective tissue, elastic</th>
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<tbody>
<tr>
<td><strong>Description:</strong> Dense regular connective tissue containing a high proportion of elastic fibers.</td>
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<tr>
<td><strong>Function:</strong> Allows recoil of tissue following stretching; maintains pulsatile flow of blood through arteries; aids passive recoil of lungs following inspiration.</td>
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<tr>
<td><strong>Location:</strong> Walls of large arteries; within certain ligaments associated with the vertebral column; within the walls of the bronchial tubes.</td>
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**Photomicrograph:** Elastic connective tissue in the wall of the aorta (250x).
C. **Reticular Fibers** - form an irregular, fine mesh work in certain tissues. They appear to have more in common with collagen than elastic fiber in that:

1. they are inelastic
2. they show the same periodic spacing (640 A) as seen in electron micrographs of collagen fiber.

Reticular fibers are the first connective tissue fibers to appear in the embryo and are still abundant in the newborn. As development proceeds, they are gradually replaced by collagenous fiber. In certain organs, reticular fibers form a mesh-work which supports the cellular components, for example, in the spleen, liver and marrow tissue.

*Figure 4.8c  Connective tissues.*

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<table>
<thead>
<tr>
<th>(c) Connective tissue proper: loose connective tissue, reticular</th>
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<tbody>
<tr>
<td><strong>Description:</strong> Network of reticular fibers in a typical loose ground substance; reticular cells lie on the network.</td>
</tr>
<tr>
<td><strong>Function:</strong> Fibers form a soft internal skeleton (stroma) that supports other cell types including white blood cells, mast cells, and macrophages.</td>
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<tr>
<td><strong>Location:</strong> Lymphoid organs (lymph nodes, bone marrow, and spleen).</td>
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*Photomicrograph:* Dark-staining network of reticular connective tissue fibers forming the internal skeleton of the spleen (350x).
GROUND SUBSTANCE - is and amorphous material surrounding the cellular and fibrous components of connective tissue. It is composed chiefly of a group of substances called proteoglycans. These compounds are synthesized by cells derived from the primitive mesenchyme, e.g., fibroblasts, chondroblasts, osteoblasts, synovial cells, smooth muscle cells and mast cells. The proteoglycans are composed of multiple polysaccharide chains covalently linked to proteins.

There are five major classes of proteoglycans:

1. Hyaluronic acid - is found in cartilage, blood vessels, skin and the umbilicus. It is the major component of synovial fluid and vitreous humor.

2. Chondroitin sulfate - found in cartilage, bone, blood vessels, skin and the cornea.

3. Dermatan sulfate - mostly in skin. Also in blood vessels, heart valves, tendons and connective tissues of the lung.

4. Keratan sulfate - found in the cornea and in cartilage.

5. Heparin sulfate - found in the aorta, liver and lung.

The chief function of these materials appears to be to help maintain the proper homeostatic environment for cells and fibrous elements.
**Loose Connective Tissues** - Primarily distinguished by the loose arrangement of its fibers.

**Types:**

I. **Mesenchyme** - a primitive, unspecialized connective tissue found abundantly in the early embryo. Gradually, it is reduced in quantity as it differentiates to form other connective tissues. Its chief components are mesenchymal cells and a ground substance which is initially fluid but becomes more fibrous with development.

II. **Mucous Connective Tissue** - is a transient tissue which is intermediate between mesenchyme and more fully differentiated tissues. As Wharton’s jelly, a chief component of the umbilicus, mucous C.T. does not differentiate further. The ingredients of this tissue include:

A. Large stellate fibroblasts along with some macrophages and lymphocytes.

B. The ground substance is soft and jelly-like giving a mucin reaction.

C. A delicate mesh of fine collagen fibers is found in the ground substance.

III. **Reticular Tissue**

**A. Composition**

1. **Cells** -
   a. Stellate cells resembling mesenchyme can be found in this tissue. These cells have a considerable amount of cytoplasm with a large pale nucleus. Cellular extensions from these cells appear to make contact with other cells.
   b. Considerable cytoplasm with a large pale nucleus.
   c. Cellular extensions appear to make contact with other cells
   d. Reticular fibroblasts are present. These cells form the reticular fiber of the tissue.
   e. Phagocytic reticular cells can be seen. These cells behave like free and fixed macrophages.

2. **Fibers** - The fibers are reticular. They are arranged as a fine lattice-work of branching threads.

**B. Occurrence** - Constitutes a significant portion of the framework of lymphoid organs, e.g., bone marrow, liver, spleen, as well as, the lamina propria of the mucous membranes of the alimentary canal and respiratory tract.

**C. Function** - Chiefly serves as a supporting framework in the organs mentioned above.
II. Areolar Connective Tissue - a loosely arranged fibroelastic connective tissue. Unstained, it appears translucent, soft and syncitial.

Figure 4.8a Connective tissues.

(a) Connective tissue proper: loose connective tissue, areolar

Description: Gel-like matrix with all three fiber types; cells: fibroblasts, macrophages, mast cells, and some white blood cells.

Function: Wraps and cushions organs; its macrophages phagocytize bacteria; plays important role in inflammation; holds and conveys tissue fluid.

Location: Widely distributed under epithelia of body, e.g., forms lamina propria of mucous membranes; packages organs; surrounds capillaries.

Photomicrograph: Areolar connective tissue, a soft packaging tissue of the body (300x).

A. Composition

1. Cells - All cell types associated with connective tissues may be seen in areolar.

   a. Fibroblasts - are the most numerous cells in areolar. The cells are large, flat and branching or spindle-shaped. The nucleus is oval and pale in stained smears. The cytoplasm is highly basophilic in young cells indicating increased protein synthesis. In older cells protein synthesis is reduced. Fibroblasts tend to be fixed except during regeneration. Then the cells exhibit slow, gliding movements.

   b. Macrophages - are most abundant in richly vascular areas. The cells are irregular with short blunt processes. Active or "angry" macrophage show numerous pseudopodia. The nucleus is ovoid and usually indented. The cytoplasm is dark staining with many vacuoles.

   c. Adipocytes or fat cells can be found scattered throughout areolar tissue. When they make up the major component of a region it is called adipose tissue. Each cell is enmeshed in a "basket" of reticular fibers.

   d. Mast cells - are found throughout connective tissues but are concentrated near blood vessels. They are easily recognized by their numerous cytoplasmic granules. These granules stain with basic dyes and PAS (periodic acid - Schiff reagent. The granules contain heparin and histamine primarily.

   e. Leukocytes - There are three types of leukocytes commonly found in areolar C.T.:

      i. Lymphocytes - are small wbcs about 7um with a large spherical nucleus. Their cytoplasm is basophilic. They can be found in abundance within the
mucous membranes of the respiratory and digestive systems. They make up the bulk of the lymphoid nodules in the lamina propria of these tracts.

ii. Eosinophiles - are granulocytic wbc's. The nucleus is bilobed. The cytoplasm contains orange acidophilic granules. Eosinophiles are especially abundant in the lamina propria of the digestive and respiratory tracts. They are associated with tissues which play a major role in allergic reactions.

iii. Plasma cells - are oblong cells whose nucleus is at one end. The nucleus shows a spoke-like pattern of chromatin. Extensive ER indicates a great deal of protein synthesis.

iv. Melanocytes - can be found in areolar tissue although their function is not well understood there. Generally, they are responsible for the manufacture of melanin.

2. Fibers - all fiber types are represented:

a. Collagenous are the most abundant.
b. Elastic fibers are not as common.
c. Reticular fibers are generally found where areolar borders on other tissues, especially near basement membranes.

3. Ground Substance - is characterized as an amorphous jelly thought to occur in sheet-like lamellae.

B. Occurrence - Areolar is the most wide spread of all connective tissues in the body.
1. It attaches epithelial layers to underlying tissues.
2. It is a general bedding substance (stoma) in the interiors of many organs.
3. Located around blood vessels, nerves and ducts.
4. It is a packing material in spaces between tissues.

C. Functions

1. It functions mechanically by:
   a. providing support with mobility
   b. increasing elasticity in tissues and organs
   c. providing pathways for blood vessels and nerves
   d. packing unused spaces in the body

2. Transport - It allows the movement of food, oxygen and wastes between capillaries and tissue cells.

3. Defense
   a. The ground substance of areolar tissue impedes the spread of pathogenic organisms except those which can digest the ground substance.
   b. Phagocytic cells in the tissue can destroy microbes.

4. Repair - The fibroblasts of areolar tissue produce new fiber and ground substance to repair damage.
III. Adipose tissue - consists of aggregations of adipocytes used as a storage tissue.

A. Composition

1. Cells - Adipocytes are highly specialized for storage. They predominate in adipose tissue:

a. Each cell is a large (120μm), clear sphere.
b. The cytoplasm is reduced to a thin shell containing a flattened nucleus surrounding an oil droplet.
c. Fat dissolves in fluids used to prepare tissues for sectioning, e.g., xylene, chloroform. Hence fat cells appear empty under the microscope. If the oil has been retained in the processing of the tissue, it can be stained specifically with Sudan III and IV (red color) or with osmium tetroxide (black).
d. Closely packed fat cells surrounded by fibrous septa make up a fat lobule supplied by a single arteriole. There is no apparent nerve supply to the lobule.
e. Various connective tissue cells can be found compressed between fat cells.

2. Fibers

a. A fine network of reticular fiber surrounds each fat cell.
b. In a fat lobule, collagenous and elastic fibers are compressed between fat cells.

3. Ground Substance is squeezed out of the lobules by the compression of fat cells.

B. Occurrence
1. Beneath the skin it forms an insulating layer not found in mammals with a hairy coat.
2. It is located around the kidneys, adrenals, mesenteries, grooves of the heart, bone marrow and in the cervical, axillary and inguinal regions.
3. It is not found in nervous tissue, lungs, eyelids and penis.

**C. Functions**

1. Stores nutritive material as neutral fat. Brown fat, a metabolically more active tissue, is only found in the human fetus.

2. Forms soft elastic pads between organs and other structures, e.g., the fat pads of the kidneys and eye balls.

3. Serves as an insulation against the cold. This is especially true in aquatic mammals where it also has a bouyancy function.

4. Esthetically, it improves the form of the body.

**Dense Fibrous Connective Tissues** - contain an abundance of compactly arranged fibers with fewer cells than in loose C.T. There is also little space for ground substance. The classification of the dense fibrous C.T.s is based on the orientation and type of fibers.

**I. Interlaced arrangement** - Fibers are arranged to withstand tensions from different directions.

**A. Predominantly Collagenous**

1. Found in deep fascias and the dermis of the skin.
2. Forms the fibrous capsules of organs like the spleen and testis.
3. Forms the sheath-like structures around bones (periosteum), muscles (epimysium) and the dura mater of the brain and spinal cord.
4. Produces the internal partitions (septa and trabeculae) of some organs.

**B. Predominently Elastic** - This form of dense C.T. can be found forming tubular sheets within arterial blood vessels.

**II. Parallel arrangement** - The fibers are oriented to withstand tension in one direction or plane.

**A. Predominently Collagenous**

1. **Tendon** - shows a very regular arrangement of fibers.
   
   a. Unit of structure is the primary tendon bundle. This structure is a collection of collagenous fibers bounded by fibroblasts.
   
   b. Bundles run in parallel but wavy courses.
   
   c. Primary bundles group together as a fascicle or secondary tendon bundle.
d. A tendon is composed of a group of fascicles.
e. The fibrous cover of a tendon is called the epitendineum.
f. Blood vessels and nerves may surround the tendon and fascicles but never enter the fascicle. This is the basis for the very slow healing of tendons.
g. The tough, inelastic tendonous sheets and cords formed by tendons provide great resistance to pulling forces, e.g., a pencil thick tendon has been found to support up to 1000 pounds.
h. Special stretch receptors in tendons help coordinate muscle action.

2. **Ligament** - has a less regular arrangement of fibers.

**B. Predominantly Elastic** - Elastic ligament

1. Elastic ligament is located in the ligamentum flava of vertebrae, the suspensory ligament of the penis, stylo-hyoid ligament, the true vocal cords and the ligamentum nuchae.
2. This elastic tissue is very yellow in color due to the abundance of elastic fibers.
3. Structurally, elastic ligament has the following characteristics:
   a. The elastic fiber is coarse (about 5μm).
   b. The elastic fibers run in parallel but branch frequently.
   c. Single fibers may be surrounded by reticular fibers.
   d. Does not show the organization of tendon.
   e. Blood vessels and nerve fibers are sparse.
4. Functionally, Elastic ligaments yield to a pulling force, then return to their original length.