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CONNECTIVE TISSUE

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Connective Tissue

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CONNECTIVE TISSUE: INTRODUCTION

The different types of connective tissue are responsible for providing and maintaining the form of organs throughout the body. Functioning in a mechanical role, they provide a matrix that connects and binds other tissues and cells in organs and gives metabolic support to cells as the medium for diffusion of nutrients and waste products. Structurally, connective tissue is formed by three classes of components: cells, fibers, and ground substance. Unlike the other tissue types (epithelium, muscle, and nerve), which consist mainly of cells, the major constituent of connective tissue is the extracellular matrix (ECM). Extracellular matrices consist of different combinations of protein fibers (collagen, reticular, and elastic fibers) and ground substance. Ground substance is a highly hydrophilic, viscous complex of anionic macromolecules (glycosaminoglycans and proteoglycans) and multiadhesive glycoproteins (laminin, fibronectin, and others) that stabilizes the ECM by binding to receptor proteins (integrins) on the surface of cells and to the other matrix components. In addition to its major structural role, molecules of connective tissue serve other important biological functions, such as forming a reservoir of factors controlling cell growth and differentiation. The hydrated nature of much connective tissue provides the medium through which nutrients and metabolic wastes are exchanged between cells and their blood supply. The wide variety of connective tissue types in the body reflects variations in the composition and amount of the cells, fibers, and ground substance which together are responsible for the remarkable structural, functional, and pathologic diversity of connective tissue.

The connective tissues originate from the mesenchyme, an embryonic tissue formed by elongated undifferentiated cells, the mesenchymal cells. These cells are characterized by oval nuclei with prominent nucleoli and fine chromatin. They possess many thin cytoplasmic processes and are immersed in an abundant and viscous extracellular substance containing few fibers. The mesenchyme develops mainly from the middle layer of the embryo, the mesoderm. Mesodermal cells migrate from their site of origin in the embryo, surrounding and penetrating developing organs. In addition to being the point of origin of all types of connective tissue cells, mesenchyme develops into other types of structures, such as blood cells, endothelial cells, and muscle cells.

1- CELLS OF CONNECTIVE TISSUE

A variety of cells with different origins and functions are present in connective tissue (Table 4–1). Fibroblasts originate locally from undifferentiated mesenchymal cells and spend all their life in connective tissue; other cells such as

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mast cells, macrophages, and plasma cells originate from hematopoietic stem cells in bone marrow, circulate in the blood, and then move into connective tissue where they remain and execute their functions. White blood cells (leukocytes) are transient cells of most connective tissues; they also originate in the bone marrow and move to the connective tissue where they reside for a few days, then usually die by apoptosis.

Lineages of connective tissue cells.

This simplified representation of the connective tissue cell lineage includes cells from the multipotential embryonic mesenchyme cells and hematopoietic stem cells of bone marrow.

Table 4–1. Functions of connective tissue cells

Cell Type	Representative Product or Activity	Representative Function
Fibroblast, chondroblast, osteoblast, odontoblast	Production of fibers and ground substance	Structural
Plasma cell	Production of antibodies	Immunologic (defense)
Lymphocyte (several types)	Production of immunocompetent cells	Immunologic (defense)
Eosinophilic leukocyte	Participation in allergic and vasoactive reactions, modulation of mast cell activities and the inflammatory process	Immunologic (defense)
Neutrophilic leukocyte	Phagocytosis of foreign substances, bacteria	Defense
Macrophage	Secretion of cytokines and other molecules, phagocytosis of foreign substances and bacteria, antigen processing and presentation to other cells	Defense
Mast cell and basophilic leukocyte	Liberation of pharmacologically active molecules (eg, histamine)	Defense (participate in allergic reactions)
Adipocyte	Storage of neutral fats	Energy reservoir, heat Production

Fibroblasts

Fibroblasts synthesize collagen, elastin, glycosaminoglycans, proteoglycans and multiadhesive glycoproteins. Fibroblasts are the most common cells in connective tissue and are responsible for the synthesis of extracellular matrix components. Two stages of activity—active and quiescent—are often observed in these cells. Cells with intense synthetic activity are morphologically

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distinct from the quiescent fibroblasts that are scattered within the matrix they have already synthesized. Some histologists reserve the term fibroblast to denote the active cell and fibrocyte to denote the quiescent cell. The active fibroblast has an abundant and irregularly branched cytoplasm. Its nucleus is ovoid, large, and pale-staining, with fine chromatin and a prominent nucleolus. The cytoplasm is rich in rough ER, and the Golgi apparatus is well developed. The quiescent fibroblast or fibrocyte is smaller than the active fibroblast and is usually spindle-shaped. It has fewer processes; a smaller, darker, elongated nucleus; and more acidophilic cytoplasm with much less RER. Fibroblasts synthesize most components of connective tissue ECM, including proteins, such as collagen and elastin, which upon secretion form collagen, reticular, and elastic fibers, and the glycosaminoglycans, proteoglycans, and glycoproteins of the ground substance. Fibroblasts are targets of various growth factors that influence cell growth and differentiation. In adults, fibroblasts in connective tissue rarely undergo division; mitosis can resume when the organ requires additional fibroblasts as in wound healing.

Adipocytes

Adipocytes are connective tissue cells that have become specialized for storage of neutral fats or for the production of heat. Often called fat cells, they have considerable metabolic significance

Macrophages & the Mononuclearphagocyte System

Macrophages were discovered and initially characterized by their phagocytic ability. They have a wide spectrum of morphologic features that correspond to their state of functional activity and to the tissue they inhabit. In the electron microscope, macrophages are characterized by an irregular surface with pleats, protrusions, and indentations, a morphologic expression of their active pinocytotic and phagocytic activities. They generally have a well-developed Golgi apparatus, many lysosomes, and rough ER. Macrophages derive from bone marrow precursor cells that divide, producing monocytes which circulate in the blood. These cells cross the wall of venules and capillaries to penetrate the connective tissue, where they mature and acquire the morphologic features of macrophages. Therefore, monocytes and macrophages are the same cell in different stages of maturation. Macrophages are also sometimes referred to as "histiocytes." Macrophages are distributed throughout the body and are present in most organs. Along with other monocyte-derived cells, they comprise a family of cells called the mononuclearphagocyte system (Table 4–2). All are long-living

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cells and may survive for months in the tissues. In most organs these cells are highly important for the up-take, processing, and presentation of antigens for lymphocyte activation. The macrophage-like cells have been given different names in different organs. However, all are derived from monocytes. The transformation from monocytes to macrophages in connective tissue involves increases in cell size, increased protein synthesis, and increases in the number of Golgi complexes and lysosomes. A typical macrophage measures between 10 and 30µm in diameter and has an oval or kidney-shaped nucleus located eccentrically.

Table 4–2 Distribution and main functions of the cells of the mononuclear phagocyte system

Cell Type	Location	Main Function
Monocyte	Blood	Precursor of macrophages
Macrophage	Connective tissue, lymphoid organs, lungs, bone marrow	Production of cytokines, chemotactic factors, and several other molecules that participate in inflammation (defense), antigen processing and presentation
Microglia cell	Nerve tissue of the central nervous system	Same as macrophages
Langerhans cell	Skin	Antigen processing and presentation
Osteoclast	Bone (fusion of several macrophages)	Digestion of bone
Dendritic cell	Lymph nodes	Antigen processing and presentation

Mast Cells

Mast cells are large, oval or round connective tissue cells, 20–30µm in diameter, whose cytoplasm is filled with basophilic secretory granules. The rather small, spherical nucleus is centrally situated and may be obscured by the cytoplasmic granules. The secretory granules are 0.3–2.0µm in diameter. Their interior is electron-dense and heterogeneous. Mast cells function in the localized release of many bioactive substances with roles in the inflammatory response, innate immunity, and tissue repair. Because of their high content of acidic radicals in their sulfated glycosaminoglycans, mast cell granules display metachromasia, which means that they can change the color of some basic dyes (eg, toluidine blue) from blue to purple or red. The granules are poorly preserved by common fixatives, so that mast cells are frequently difficult to identify. Mast cell granules contain a wide variety of paracrine compounds that promote

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different aspects of a local inflammatory response. A partial list of important molecules released from these granules includes:

- **Heparin**, a sulfated glycosaminoglycan that acts locally as an anticoagulant
- **Histamine**, which promotes increased vascular permeability and smooth muscle contraction
- **Serine proteases**, which activate various mediators of inflammation
- **Eosinophil & neutrophil chemotactic factors** which attract those leukocytes
- **Leukotrienes C4, D4, and E4** (or the slow-reacting substance of anaphylaxis, SRS-A) which also trigger smooth muscle contraction

Plasma Cells

Plasma cells are large, ovoid cells that have a basophilic cytoplasm due to their richness in rough ER. The juxtannuclear Golgi apparatus and the centrioles occupy a region that appears pale in regular histologic preparations. The nucleus of the plasma cell is generally spherical but eccentrically placed. Many of these nuclei contain compact, peripheral regions of heterochromatin alternating with lighter areas of euchromatin, a configuration that can give the nucleus of a plasma cell the appearance of a clock-face. There are few plasma cells in most connective tissues. Their average lifespan is short, 10–20 days.

Leukocytes

Connective tissue normally contains leukocytes that migrate from the blood vessels by diapedesis. Leukocytes or white blood corpuscles, are the wandering cells of the connective tissue. They leave blood by migrating between the endothelial cells lining capillaries and postcapillary venules to enter connective tissue by a process called diapedesis. This process increases greatly during inflammation, which is a vascular and cellular defensive reaction against foreign substances, in most cases pathogenic bacteria or irritating chemical substances. The classic signs of inflammation were first described by Celsus in the first century as redness and swelling with heat and pain (rubor et tumor cum calore et dolore). Inflammation begins with the local release of chemical mediators of inflammation, substances of various origin (mainly from local cells and blood plasma proteins) that induce some of the events characteristic of inflammation, eg, increase of blood flow and vascular permeability, chemotaxis, and phagocytosis.

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2- FIBERS OF CONNECTIVE TISSUE

The connective tissue fibers are formed by proteins that polymerize into elongated structures. The three main types of connective tissue fibers are collagen, reticular, and elastic fibers. Collagen and reticular fibers are both formed by the protein collagen, and elastic fibers are composed mainly of the protein elastin. These fibers are distributed unequally among the types of connective tissue and the predominant fiber type is usually responsible for conferring specific properties on the tissue.

Collagen

The collagens constitute a family of proteins selected during evolution for the execution of several (mainly structural) functions. During the process of evolution of multicellular organisms, a family of structural proteins was selected by both environmental influences and the functional requirements of the animal organism and developed to acquire varying degrees of rigidity, elasticity, and strength. These proteins are known collectively as collagen, and the chief examples among its various types are present in the skin, bone, cartilage, smooth muscle, and basal lamina. Collagen is the most abundant protein in the human body, representing 30% of its dry weight. The collagens are produced by several cell types and are distinguishable by their molecular compositions, morphologic characteristics, distribution, functions, and pathologies. More than 20 types of collagen have been identified and designated with Roman numerals.

Collagen synthesis.

Hydroxylation and glycosylation of procollagen chains and their assembly into triple helices occurs in the RER and further assembly into fibrils occurs in the ECM after secretion of procollagen. Because there are many slightly different genes for procollagen chains and collagen production depends on several post-translational events involving several other enzymes, many diseases involving defective collagen synthesis have been described. The other fibrillar collagens are formed in processes similar to that described for collagen type I. In summary, collagen synthesis involves a cascade of unique post-translational modifications of the original procollagen polypeptides. All these modifications are critical to the structure and function of normal mature collagen. Because there are so many steps in collagen biosynthesis, there are many points at which the process can be interrupted or changed by defective enzymes or by disease processes. Although fresh collagen fibers are colorless strands, when present in large number (eg, in tendons) they appear white. The highly regular orientation of subunits in collagen fibers makes them birefringent under the polarizing microscope. In the light microscope, collagen fibers are acidophilic; they stain pink with eosin, blue with

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Mallory trichrome stain, green with Masson trichrome stain, and red with Sirius red. Because of the long and tortuous course of collagen bundles, their length and diameter are better studied in spread preparations than in histologic sections. Mesentery is frequently used for this purpose; when spread on a slide, this structure is sufficiently thin to let the light pass through; it can be stained and examined directly under the microscope. Mesentery consists of a central portion of connective tissue lined on both surfaces by a simple squamous epithelium, the mesothelium. The collagen fibers in a spread preparation appear as elongated and tortuous cylindrical structures of indefinite length, with a diameter that varies from 1 to 20 μ m.

Reticular Fibers

Once thought to be uniquely distinct from collagen, reticular fibers are now known to consist mainly of collagen type III, which forms extensive networks of extremely thin (diameters 0.5–2 μ m) and heavily glycosylated fibers in certain organs. They are not visible in hematoxylin-and-eosin (H&E) preparations but can be easily stained black by impregnation with silver salts. Because of their affinity for silver salts, these fibers are called argyrophilic. Reticular fibers are also periodic acid–Schiff (PAS)-positive, which like argyrophilia is due to the high content of sugar chains associated with these fibers. Reticular fibers contain 6–12% hexoses as opposed to 1% in most collagen fibers. Reticular fibers constitute a network around the parenchymal cells of various organs (eg, liver, endocrine glands) and are particularly abundant in the framework of hematopoietic organs (eg, spleen, lymph nodes, red bone marrow). In the latter sites the network is produced by fibroblast-like cells called reticular cells. The loose disposition of reticular fibers creates a flexible network in these organs and others that are subject to changes in form or volume, such as the arteries, uterus, and intestinal muscle layers.

Elastic Fibers

Elastic fibers are also thinner than the average collagen fiber and form sparse networks interspersed with collagen bundles in many organs subject to much bending or stretching, such as the wall of large arteries. The name indicates the major functional property such fibers impart to these resilient organs. Elastic fibers develop through successive stages. In the first stage, a core of 10-nm microfibrils forms from several different glycoproteins, notably the large glycoprotein called fibrillin. Fibrillin binds elastin and forms the scaffolding necessary for the deposition of elastin. Defective fibrillin results in the formation of fragmented elastic fibrils. In the second stage of development, the protein elastin is deposited between the microfibrils, forming larger fibers. During the

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third stage, elastin gradually accumulates until it comprises most of the fiber bundles, which are further surrounded by a thin sheath of microfibrils. These are the mature elastic fibers, the most numerous component of the elastic fiber system. Stages of elastic fiber formation are shown in. In the wall of large blood vessels, especially arteries, elastin also occurs as fenestrated sheets called elastic lamellae

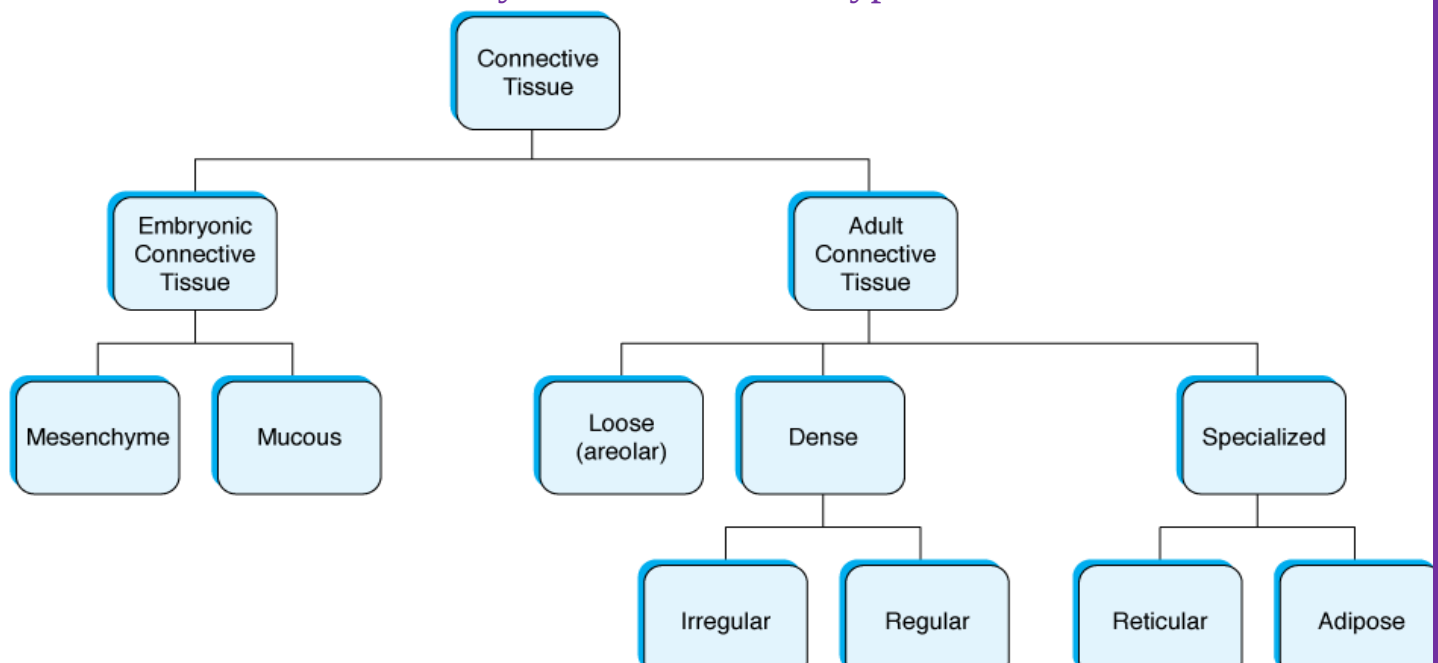
3- GROUND SUBSTANCE OF CONNECTIVE TISSUE

The ground substance of the ECM is a highly hydrated, transparent, complex mixture of macromolecules, principally in three classes: glycosaminoglycans (or GAGs), proteoglycans, and multiadhesive glycoproteins. The complex molecular mixture of the ground substance is transparent and rich in bound water. It fills the space between cells and fibers of connective tissue and, because it is viscous, acts as both a lubricant and a barrier to the penetration of invaders. When adequately fixed for histologic analysis, its components aggregate and precipitate in the tissues as granular material that is observed in TEM preparations as electron-dense filaments or granules

TYPES OF CONNECTIVE TISSUE

Connective tissues composed of the cells, fibers, and ground substance components already described nevertheless are quite variable in histological structure. This has led to the use of descriptive names or classifications for various connective tissue types, denoting either the major component or a structural characteristic of the tissue.

Classification commonly used for the main types of connective tissue.



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Loose connective tissue

Is a very common type of connective tissue that supports many structures which are normally under some pressure and low friction. It usually supports epithelial tissue, forms a layer around small blood and lymphatic vessels, and fills the spaces between muscle and nerve fibers. Loose connective tissue is also found in the papillary layer of the dermis, in the hypodermis, in the linings of the peritoneal and pleural cavities, in glands, and in the mucous membranes (wet membranes that line the hollow organs) supporting the epithelial cells. Loose connective tissue, sometimes called **areolar tissue**, has all the main components of connective tissue (cells, fibers, and ground substance) in roughly equal parts. The most numerous cells are fibroblasts and macrophages, but other types of connective tissue cells are also present. Collagen, elastic, and reticular fibers also appear in this tissue. With a moderate amount of ground substance, loose connective tissue has a delicate consistency; it is flexible, well vascularized, and not very resistant to stress.

Dense connective tissue

Is adapted to offer resistance and protection. It has the same components found in loose connective tissue, but there are fewer cells and a clear predominance of collagen fibers over ground substance. Dense connective tissue is less flexible and far more resistant to stress than is loose connective tissue. It is known as **dense irregular** connective tissue when the collagen fibers are arranged in bundles without a definite orientation. The collagen fibers form a 3-dimensional network in dense irregular tissue, providing resistance to stress from all directions. Dense irregular connective tissue is often found closely associated with loose connective tissue. The two types frequently grade into one another and distinctions between them are often arbitrary. The collagen bundles of **dense regular** connective tissue are arranged according to a definite pattern, with collagen fibers aligned with the linear orientation of fibroblasts in response to prolonged stresses exerted in the same direction. This arrangement offers great resistance to traction forces. Tendons and ligaments are the most common examples of dense regular connective tissue. These elongated cylindrical structures hold together components of the musculoskeletal system; by virtue of their richness in collagen fibers, they are white and inextensible. They have parallel, closely packed bundles of collagen separated by a very small quantity of ground substance. Their fibrocytes contain elongated nuclei parallel to the fibers and sparse cytoplasmic folds that envelop portions of the collagen bundles. The cytoplasm of these fibrocytes is rarely revealed in H&E stains, not only because it is sparse but also because it stains the same color as the fibers. Some ligaments,

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such as the yellow ligaments of the vertebral column, also contain abundant parallel elastic fiber bundles. The collagen bundles of tendons vary in size and are enveloped by small amounts of loose connective tissue containing small blood vessels and nerves. Overall, however, tendons are poorly vascularized and repair of damaged tendons is very slow. Externally, the tendon is surrounded by a sheath of dense irregular connective tissue. In some tendons, this sheath is made up of two layers, both lined by flattened **synovial cells** of mesenchymal origin. One layer is attached to the tendon, and the other lines neighboring structures. The space between these linings contains a viscous fluid (similar to the fluid of synovial joints) composed of water, proteins, hyaluronate and other GAGs. This synovial secretion acts as a lubricant permitting easy sliding movements of the tendon within its sheath.