General Connective Tissue

1. Loose Areolar. Collagen fibers (purple), elastic fibers (black), fat cell (F).
2. Reticular fibers of liver stained black with silver.
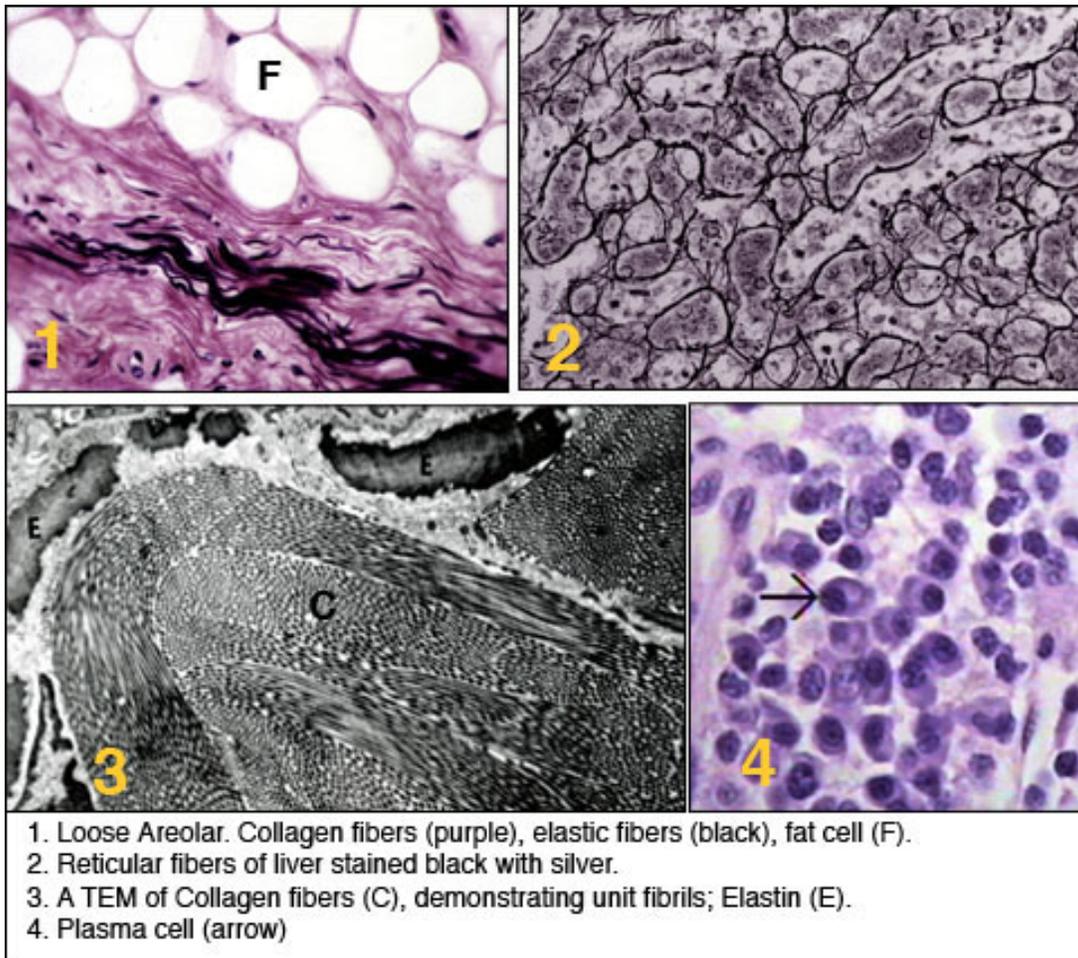3. A TEM of Collagen fibers (C), demonstrating unit fibrils; Elastin (E).
4. Plasma cell (arrow)

Connective tissues form a diverse group of tissues derived from mesenchyme and, in keeping with the diversity of types, have a number of different functions. They provide structural elements, serve as a foundation for the support of organs, form a packing material for otherwise unoccupied space, provide an insulating layer (fat) that also acts as a storage depot that can be used to provide energy, and play a vital role in the defense mechanisms of the body and in repair after injury. Some are functions of ordinary (general) connective tissues; others are functions of specialized connective tissues. In organs, connective tissues form the stroma and epithelial (cellular) components make up the parenchyma.

Organization

Like all basic tissues, connective tissues are made up of cells and intercellular substances. The latter consist of fibers, ground substance, and tissue fluid. Unlike epithelium, where the cells are closely apposed with little intercellular material, connective tissue cells are widely separated by the intercellular fibers and ground substance that form the bulk of these tissues. Together, the fibers and ground substance form the matrix of connective tissue. With some exceptions, connective tissue is generally well vascularized.

Classification

Classification of connective tissues into various subgroups is largely descriptive of extracellular materials rather than features of the cellular components. Two large categories can be defined - general and special - from which further subdivisions are made. General connective tissues are distinguished as loose or dense according to whether the fibers are loosely or tightly packed. Loose connective tissues can be subdivided on the basis of some special properties of their constituents, such as adipose (fatty) tissue, reticular tissue, and so forth. Dense connective tissues can be subdivided according to whether the fibers are randomly distributed or show an orderly arrangement. Thus, dense connective tissues are classed as dense irregular or dense regular connective tissues.

Loose Connective Tissue

Loose connective tissue is a common and simple form of connective tissue and can be considered the prototype of connective tissue. Essentially all other types of connective tissues are variants of loose connective tissue in which one or more components have been emphasized to serve specific functions.

Fibers of Connective Tissue

The fibrous intercellular substances in connective tissue consist primarily of collagenous, reticular, and elastic fibers. Collagen fibers are present in all connective tissues and run an irregular course with much branching. The fibers vary in thickness from 1 to 10 μm and are of undefined length. Although flexible, collagenous fibers have extremely high tensile strength. Ultrastructurally, the fibers consist of parallel fibrils, each of which represents a structural unit. These unit fibrils show repeating transverse bands spaced at 64-nm intervals along their length and are composed of macromolecules of tropocollagen that measure about 260 nm long by 1.5 nm wide. The tropocollagen molecules lie parallel to each other, overlapping by about one-fourth their lengths; the overlap is responsible for the banding pattern. Each molecule of tropocollagen consists of three polypeptide chains called alpha units arranged in a helix and linked by hydrogen bonds. The polypeptides are rich in glycine and proline and also contain hydroxyproline and hydroxylysine. However, alpha units isolated from collagens taken from different sites vary somewhat in the composition and sequence of amino acids. Several distinct molecular types of collagen have been identified, all of which consist of three alpha units arranged in a right hand helix and differ mainly in the amino acid constituents of the alpha chain. Not all unit fibrils of the various collagens present a banded, fibrillar appearance, nor are they arranged in a similar fashion.

Thus, collagen represents a family (in excess of 12 types) of closely related but genetically distinct proteins. Tropocollagen has been extracted from the ground substance also but cannot be demonstrated histologically. Cells other than fibroblasts can make various types of collagen, and collagen is synthesized by chondroblasts, osteoblasts, odontoblasts, epithelial cells, endothelial cells and smooth muscle cells. Collagen molecules are important components of basement membranes.

Reticular fibers do not gather into large bundles as do collagenous fibers but tend to form delicate networks. They are not seen in routinely prepared sections but can be demonstrated with silver stains or by the periodic acid-Schiff (PAS) reagent, which reacts with proteoglycans that bind reticular unit fibrils together. In electron micrographs, the unit fibrils of reticular fibers

show the same banding pattern as the unit fibrils of type I collagen fibers. They differ structurally in number, diameter, and in the arrangement of the unit fibrils. The unit fibrils of reticular fibers consist of type III collagen.

Elastic fibers appear as thin, homogeneous strands that are similar and of more uniform size than collagen fibers. They cannot be distinguished readily in routine H&E sections and require special stains to make them easily visible. In electron micrographs, elastic fibers are seen to consist of bundles of microfibrils embedded around an amorphous component called elastin. The microfibrils are about 11 nm in diameter and lack cross banding. During formation of elastic fibers, microfibrils are laid down first and elastin is added secondarily but soon forms the bulk of the fiber. A precursor molecule, tropoelastin, is released by cells and polymerizes extracellularly to form elastin. The microfibrillar component tends to be peripherally located on the fiber. Elastin, like collagen, contains the amino acids glycine and proline but has little hydroxyproline and lacks hydroxylysine. It has a high content of valine and contains two amino acids, desmosine and isodesmosine, that are specific to elastin. Microfibrils consist of a nonsulfated glycoprotein called fibrillin. Similar microfibrils also exist that are associated with the basal lamina of epithelia. Fibrillin mediates adhesion between elastin and the other components of the extracellular matrix. Elastic fibers can stretch more than 130% of their original length, and their presence permits connective tissues to undergo considerable expansion or stretching with return to the original shape or size when the deforming force is removed. In addition to forming fibers, elastin may be present in fenestrated sheets or layers called elastic laminae in the walls of blood vessels.

Ground Substance

The fibers and cells of connective tissue are embedded in an amorphous material called ground substance that is present as a transparent gel of variable viscosity. Ground substance consists of proteoglycans, glycosaminoglycans, and glycoproteins that differ in amount and type in different connective tissues. Proteoglycans have a bottlebrush configuration with long protein cores bound covalently to numerous glycosaminoglycan side chains. Glycosaminoglycans are linear polymers of repeating disaccharide units that contain hexosamine (N-acetylglucosamine, N-acetylgalactosamine), D-glucosamine, D-galactosamine, or uronic (glucuronic) acid as their most constant feature. Because these macromolecules of the ground substance are commonly sulfated (SO_3^-) or in the case of uronic acid which exhibits a carboxyl group (Coo^-) they impart a net negative charge to the molecule. As a result, these molecules have a strong hydrophilic behavior and are instrumental in the retention of positive ions (Na^+) together with water in the extracellular matrix. Thus, these large, negatively charged molecules form and maintain a large hydration space (an extracellular fluid compartment) within the extracellular matrix. As a result of these molecules, the ground substance contains a high proportion of water, which is bound to long-chain carbohydrates and proteoglycans; most of the extravascular fluid (tissue fluid) is in this state. This bound water acts as a medium by which nutrients, gases, and metabolites can be exchanged between blood and tissue cells. Hyaluronic acid is the chief glycosaminoglycan of loose connective tissue, and because of its ability to bind water, it is primarily responsible for changes in the permeability and viscosity of loose connective tissue. In addition, proteoglycans play an important role in maintaining the structural organization of the fibrous constituents in the matrix and basal laminae and aid in preventing or retarding the spread of microorganisms and their toxic materials from the site of an infection. The primary glycoproteins of the matrix are fibronectin, laminin, and thrombospondin. These are adhesion glycoproteins that function to link cell membranes of

various cell types to elements comprising the extracellular matrix. Laminin is the largest of these adhesion glycoproteins and is most abundant in the lamina lucida of epithelial basal laminae and in the external laminae of muscle cells. It binds the plasmalemma via a transmembrane glycoprotein laminin receptor of epithelial and muscle cells to type IV collagen and proteoglycans (heparan sulfate) in the adjacent matrix. Fibronectin is a large, flexible glycoprotein of the extracellular matrix synthesized by fibroblasts and some epithelia. It also occurs in the external laminae of muscle cells and Schwann cells as well as on the surfaces of other cell types. Fibronectin mediates adhesion between cells and between cells and substrates in the matrix. It has several receptor-binding domains and links the plasmalemma of epithelial cells to collagen fibers and glycosaminoglycans. Through fibronectin receptors (integrins) in the basal epithelial plasmalemma, fibronectin links actin filaments within the cell cytoplasm to components of the extracellular matrix. Fibronectin is thought to be involved in cell spreading and locomotion. Thrombospondin is another adhesive glycoprotein that binds to collagen and some proteoglycans. It is produced by fibroblasts, smooth muscle cells, and epithelial cells and binds the plasmalemma of these cell types to the extracellular matrix.

Cells of Connective Tissue

Connective tissue contains several different cell types. Some are indigenous to the tissues; others are transients derived from blood. The most common connective tissue cells are fibroblasts - large, flattened cells with elliptical nuclei that contain one or two nucleoli. The cell shape is irregular and often appears stellate with long cytoplasmic processes extending along the connective tissue fibers. The boundaries of the cell are not seen in most preparations, and the morphology varies with the state of activity. In active cells the nuclei are plump and stain lightly, whereas the nuclei of inactive cells appear slender and dense. Ultrastructurally, active cells show increased amounts of granular endoplasmic reticulum (GER). Fibroblasts elaborate the precursors of collagen, reticular and elastic fibers, produce the ground substance, and maintain these extracellular materials that are constantly being remodeled and renewed. This fact explains conditions such as scurvy that results from a deficiency of vitamin C in the diet. Vitamin C is necessary for proper cross-linking of the molecules that make up the collagen fibers and the lack of it results in weakened collagen and connective tissue throughout the body.

The peptides of collagen are formed on the ribosomes of the GER, from which they are transported to the Golgi complex. Procollagen, a molecular form of collagen is released at the maturing face of the Golgi complex within secretory vesicles into the surrounding cytoplasm and then released from the cell by exocytosis. An extracellular enzyme called procollagen peptidase converts the procollagen molecules into tropocollagen, which then polymerize extracellularly to form the unit fibrils of collagen.

Myofibroblasts resemble fibroblasts but contain aggregates of the contractile microfilaments, actin and myosin. In contrast to smooth muscle cells, myofibroblasts lack a surrounding external lamina. Normally found only in small numbers, they increase following tissue injury. Myofibroblasts produce collagen, and their contractile activity contributes to the retraction and shrinkage of early scar tissue.

Macrophages (histiocytes) are almost as abundant as fibroblasts in areolar connective tissue. They are actively phagocytic, ingesting a variety of materials from particulate matter to bacteria, tissue debris, and whole dead cells. The material ingested is broken down by lysosomal digestion. Macrophages are activated by lipopolysaccharides (a surface component

of gram-negative bacteria) and interferon-gamma. The effectiveness of macrophages is enhanced by the binding of complement and antibodies to the surface of bacteria (opsonization). Opsonized bacteria are more vulnerable to phagocytosis. Complement is a group of proteins circulating in the blood plasma that are synthesized and released by the liver. C3 (a component of complement) promotes the lysis and/or phagocytosis of microbes by binding to their cell membranes. Fc antibody receptor in the macrophage plasmalemma binds to the coated bacteria and phagocytoses them for lysosomal digestion. Some invasive materials (asbestos, bacilli of tuberculosis,Toxoplasma) do not undergo lysosomal digestion and in response macrophages fuse together to form foreign body giant cells. Macrophages also interact with lymphocytes by releasing interleukin-6 (stimulates the differentiation of B lymphocytes into plasma cells) and interleukin-1 (stimulates T lymphocytes to divide) to combat infections. Macrophages can synthesize and release a number of other factors such as pyrogens (factors that mediate fevers), granulocyte-macrophage colony-stimulating factor, fibroblast growth factor, transforming growth factor, and tumor necrosis factor, as well as connective tissue enzymes such as collagenase and elastase. Macrophages commonly are described as irregularly shaped cells with blunt cytoplasmic processes and ovoid or indented nuclei that are smaller and stain more deeply than those of fibroblasts. In fact, macrophages are difficult to distinguish morphologically from fibroblasts, especially active fibroblasts, unless the macrophages show evidence of phagocytosis. The macrophages of loose connective tissue are part of a widespread system of mononuclear phagocytes that includes phagocytes of the liver (Kupffer cells), lung (alveolar macrophages), serous cavities (pleural and peritoneal macrophages), nervous system (microglia), lymphatic tissue, and bone marrow. Regardless of where they are found, macrophages have a common origin from precursors in the bone marrow, and the monocytes of blood represent a transit form of immature macrophages.

Mast cells are present in variable numbers in loose connective tissue and often collect along small blood vessels. They are large, ovoid cells 20 to 30 μm in diameter with large granules that fill the cytoplasm. Two populations of mast cells are known to exist: connective tissue mast cells and mucosal mast cells. In humans, the granules of connective tissue mast cells are membrane-bound and in electron micrographs show a characteristic tubular pattern. The granules contain heparin, a potent anticoagulant; histamine, an agent that causes smooth muscle contraction in bronchi and increased vascular permeability; leukotriene C4 and D4, which increase vascular permeability, increases vasodilation, and causes smooth muscle contraction in bronchi; and eosinophil chemotactic factor. This factor attracts eosinophils to the inflammation site. The granules of mucosal mast cells contain chondroitin sulfate rather than heparin. Mast cells have highly specific membrane receptors for the Fc segment of IgE produced in response to allergens. Both mast cell types arise from bone marrow stem cells. Mast cells are especially numerous along small blood vessels and beneath the epithelia of the intestinal tract and respiratory system. Here, they detect the entry of foreign proteins and initiate a local inflammatory response by rapidly discharging their secretory granules. The discharge of granules is unique in that several fuse together and release their contents simultaneously. Mast cells also can promote immediate hypersensitivity reactions (hay fever, asthma, and anaphylaxis) following their release of secretory granules, which act as chemical mediators.

Fat cells are specialized for synthesis and storage of lipid. Individual fat cells may be scattered throughout loose connective tissue or may accumulate to such an extent that other cells are crowded out and an adipose tissue is formed. Each fat cell acquires so much lipid that the nucleus is flattened to one side of the cell and the cytoplasm forms only a thin rim around a

large central droplet of lipid. In ordinary H&E sections, fat cells appear empty due to the loss of lipid during tissue preparation, and groups of fat cells have the appearance of a honeycomb.

Plasma cells are not common in most connective tissues but may be numerous in the lamina propria of the gastrointestinal tract and are present in lymphatic tissues. Plasma cells appear somewhat ovoid, with small, eccentrically placed nuclei in which the heterochromatin is arranged in coarse blocks to form a "clock face" pattern. The cytoplasm is basophilic due to the abundance of GER. A weakly staining area of cytoplasm called the negative Golgi image often appears adjacent to one side of the nucleus and corresponds to the site of the Golgi complex. Plasma cells produce immunoglobulins (antibodies) that form an important defense against infections. Various acidophilic inclusions may be found in the cytoplasm of some plasma cells. Plasma cells are a differentiated form of the B-lymphocyte. Variable numbers of leukocytes constantly migrate into the connective tissues from the blood (diapedesis).

Neutrophils are one type of leukocyte characterized by a lobed nucleus. In this cell type, the granules are said to be neutrophilic, although in sections they stain faintly pink (i.e., acidophilic). The cells are avidly phagocytic for small particles and bacteria and are especially numerous at sites of infection. Neutrophils also contain antioxidants to destroy potentially toxic peroxides generated during lysosomal activity. Once neutrophils are activated in tissues they do not live long and die. They form the major cellular component of the substance called pus.

Eosinophils also are characterized by lobed nuclei, but their cytoplasmic granules are larger, more spherical, and more discretely visible than those of neutrophils. The granules stain intensely with acid dyes such as eosin. All eosinophils have surface receptors for IgE. These cells are phagocytic for specific molecules and have a special avidity for antigen-antibody complexes. They increase in number as a result of allergic or parasitic types of disease. Eosinophils are attracted to the sites of histamine release by eosinophilic chemotactic factor of anaphylaxis (ECF-A), and their cytoplasmic granules contain enzymes capable of breaking down histamine. Eosinophils also produce major basic protein, which enhances the antibody-mediated destruction of the larvae of some helminthic parasites.

Lymphocytes belong to the class of leukocytes called mononuclear leukocytes and are characterized by a single, round, non-lobed nucleus. They are the smallest of the cells that migrate into connective tissues and measure about 7 μm in diameter. These cells form part of the immunologic defense system and may give rise to antibody-producing cells (B lymphocytes) or elaborate nonspecific factors that destroy foreign cells (T lymphocytes). B and T lymphocytes cannot be distinguished morphologically using routine H&E preparations. They are few in number in normal connective tissue but increase markedly in areas of chronic inflammation.

Monocytes are the blood-borne forerunners of tissue macrophages. Once these cells have entered connective tissue, it is difficult, if not impossible, to distinguish them from macrophages. As monocytes leave the vasculature and become macrophages they receive further specific, molecular programming dependent on the microenvironment (spleen, lung, brain) within which they reside. Some macrophages have antigen-presenting functions and these specialized macrophages form a family of antigen presenting cells. This type of macrophage phagocytoses endogenous antigens that are degraded into antigen peptide fragments. These fragments are then presented on the macrophage surface together with class II major histocompatibility complex (MCH). The presented antigen peptide fragment is recognized by CD4+ helper T lymphocytes, which are stimulated and in turn promote B lymphocyte differentiation.

Subtypes of Loose Connective Tissue

Mesenchyme is a loose, spongy tissue that serves as packing between the developing structures of the embryo. It consists of a loose network of stellate and spindle-shaped cells embedded in an amorphous ground substance with thin, sparse fibers. The cells have multiple developmental potentials and can give rise to any of the connective tissues. The presence of mesenchymal cells in the adult often has been proposed to explain the expansion of adult connective tissue. However, fibroblasts, too, are capable of sequential divisions, and may fulfill this role.

Mucoid tissue occurs in many parts of the embryo but is particularly prominent in the umbilical cord, where it is called Wharton's jelly. It resembles mesenchyme in that the constituent cells are stellate fibroblasts with long processes that often make contact with those of neighboring cells, and the intercellular substance is soft and jelly-like and contains thin collagen fibers. The fiber content increases with the age of the fetus. Mucoid tissue does not have the developmental potential of mesenchyme.

Areolar connective tissue is a loosely arranged connective tissue that is widely distributed in the body. It contains collagen fibers, reticular fibers, and a few elastic fibers embedded in a thin, almost fluid-like ground substance. This kind of connective tissue forms the stroma that binds organs and organ components together. It forms helices about the long axes of expandable tubular structures such as the ducts of glands, the gastrointestinal tract, and blood vessels.

Adipose Tissue

Adipose tissue differs in two respects from other connective tissue: fat cells and not the intercellular substances predominate, and unlike other connective tissue cells, each fat cell is surrounded by its own basal lamina. Reticular and collagenous fibers also extend around each fat cell to provide a delicate supporting framework that contains numerous capillaries. Adipose tissue can be subdivided into white and brown fat. White fat is the more plentiful and is found mainly in the subcutaneous tissue (where it forms the panniculus adiposus), omenta, mesenteries, pararenal tissue, and bone marrow. White fat is an extremely vascular tissue and also contains many nerve fibers from the autonomic nervous system. In white fat, the cells are filled by a single, large droplet of lipid; thus, it is often referred to as unilocular fat. The lipid droplet is composed of glycerol esters and fatty acids. The materials within the fat droplet are in a constant state of flux and are not permanent entities. Adipocytes have receptors for insulin, thyroid hormone, glucocorticoids, and norepinephrine that modulate the release and uptake of lipid. White fat serves as a storage depot for calories taken in excess of the body's needs and can be used for production of energy as required or as a source of building materials as in the case of steroid hormone synthesis. It also acts as an insulating layer to conserve body heat, acts mechanically as a packing material, and forms shock-absorbing pads in the palms of the hands, on the soles of the feet, and around the eyeballs. Adipocytes secrete a hormone called leptin the action of which is to decrease appetite. This action is thought to be mediated through satiety centers in the hypothalamic region of the brain where leptin receptors are found.

Brown fat is present in many species and is prominent in hibernating animals and newborn humans. Brown fat has a restricted distribution, occurring mainly in the interscapular and inguinal regions. The brown or tan color is due to the high content of cytochrome enzymes. The cells show round nuclei, and the cytoplasm is filled with multiple small droplets of lipid;

hence this type of fat is called multilocular fat. Mitochondria here are more numerous and larger than those in the cells of white fat. Each cell of brown fat receives direct sympathetic innervation. During arousal from hibernation in animals or following birth in the case of humans, the lipid within the brown fat is rapidly oxidized to produce heat and release substances such as glycerol that are used by other tissues. The energy produced by mitochondria in brown fat is dissipated as heat rather than being stored as ATP. Because brown fat is even more vascular than white fat, the generated heat raises the temperature of the blood significantly, thus increasing the general body temperature.

Reticular Connective Tissue

Reticular connective tissue is characterized by a cellular framework as seen in lymphatic tissues and bone marrow. Reticular cells are stellate, with processes extending along the reticular fibers to make contact with neighboring cells. The cytoplasm stains lightly and is attenuated, and the large nucleus stains weakly. The reticular cell is equivalent to the fibroblast of other connective tissues and is responsible for the production and maintenance of the reticular fibers, which are identical to those found in loose connective tissue.

Dense Connective Tissue

Dense connective tissue differs from the loose variety chiefly in the concentration of fibers and reduction of the cellular and amorphous constituents. Two types, dense irregular and dense regular, are identified.
Dense irregular connective tissue contains abundant, thick, collagenous bundles that are woven into a compact network. Among the collagen fibers is an extensive network of elastic fibers. An example of dense irregular connective tissue is the dermis of the skin. Dense regular connective tissue also contains a predominance of collagen fibers arranged in bundles, but these have a regular, precise arrangement. The organization of the collagen bundles reflects the mechanical needs of the tissue. In tendons, aponeuroses, and ligaments, for example, they are oriented in the direction of pull. Fibroblasts are the primary cells present and occur in rows parallel to the bundles of collagen fibers.

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