ANAT 2160/BIOL 3430 Connective Tissue and Muscle Laboratory Module

1) Connective tissue

Objectives:
1) identify the components (cells, fibres) present in "ordinary" connective tissue
2) differentiate the three types of ordinary connective tissue in the body, based on their fibre content.

Overview
Connective tissue is the basic tissue type that provides structural and metabolic support for the other tissues in the body. There are many kinds of connective tissue; in this laboratory exercise we will be examining "ordinary" connective tissue (sometimes called "proper " connective tissue), which is widespread throughout the body. The specialized types of connective tissue (bone, cartilage, blood) will be the subjects of other laboratory exercises. Here you will be looking at the cells and fibres generally present in ordinary connective tissue, and will see the differences between loose and dense connective tissue, and between regular and irregular dense connective tissue.

A) Ground substance: ground substance, making up the bulk of the extracellular matrix of connective tissue, is composed of hydrated macromolecules which cannot be vizualized using the stains normally used to show up the components of tissues. You will therefore not see any slides stained specifically to show ground substance in this laboratory exercise, but you should be aware that it is present in all types of connective tissue.

B) Cells of connective tissue. These cell types are common to all ordinary connective tissue.

Fibroblasts. In developing tissues fibroblasts have large, oval nuclei and large, branched cytoplasm because they are actively secreting the macromolecules that will make up their surrounding ground substance and fibres. However in adult tissues these cells play a much-reduced tissue maintenance role so are generally not very active (in fact they can be called fibrocytes, but the term "fibroblasts" is still widely used). The nuclei of adult fibroblasts are relatively thin, elongated and may be bent. The cytoplasm is thin with and elongated extensions that run between fibres in the extracellular matrix. These extensions are too thin to be seen in the light microscope. The presence of fibroblasts in tissues is judged by looking for the characteristic nuclear shapes seen in the slides below.
26, 68, skin. Look deep to the epidermis (the outer, darker-staining, highly cellular epithelial layer of the skin), in the dermis, where two kinds of ordinary connective tissue can be identified (see below, section D). Collagen fibres stain light pink on these slides; look between the collagen fibres for the thin, flat, tapering nuclei of the fibroblasts; these nuclei stain very darkly. How does the number of fibroblast nuclei vary in the different regions of the dermis? Is this variation correlated with the amount of collagen present?

Macrophages. These cells are mobile scavengers that go by various names in different organs in the body (for instance, in the liver they are called Kupffer cells). Their function is to phagocytose invading pathogens. In the respiratory system these cells collect foreign objects such as dust and smoke particles that are inspired into the lungs.
59, lung. This slide consists mostly of empty space (the airways) with thin pink-staining sheets of tissue dividing up the interior. Scattered among the airway walls are dirty-looking patches with small local collections of brown particles; these particles are dust collected by macrophages. Scan the slide slowly at the lowest magnification to find aggregates of particle-containing macrophages, then use the higher power lenses to see if you can find the nuclei of these cells (usually round or oval, pale purple). You may only be able to see the dust particles themselves within the macrophage cytoplasm if the staining on your slide is not strong.

Adipocytes. The large volume of cytoplasm in these cells is made up of lipids, so the nuclei of these cells are thin, flat and pushed against one side of the cells. When fatty tissue is prepared for histology, the lipids are dissolved away so the cells appear to be large, empty circular or oval structures. Fat in connective tissue provides padding against mechanical shocks and also serves as a packing material to fill space within organs and tissues in the body.
54, adult thymus. The thymus gland is where T-cells (a type of lymphocyte, or white blood cell, belonging to the immune system) mature before being released into the circulation to help fight infections and invading pathogens. This gland is most active in infants, but the amount of active immune tissue becomes reduced in adolescence and adulthood, so that the active tissue becomes replaced with adipocytes. Look for a single thin, flat darkly staining nucleus around the edge of each of these adipocytes.

Mast cells. When activated by local allergens or chemicals, these cells release histamine and other defence-related compounds from granules within the cells. The granular vesicles containing histamine are darkly stained.
11, thyroid gland. The thyroid follicles are large round or oval structures lined with a simple cuboidal epithelium, making up most of the tissue in this slide. The pinkish-staining material contained in the follicles is a precursor for thyroid hormone. To identify mast cells, look for small, round, elongated or irregularly shaped cells with their cytoplasm filled with dark purple granular material, located in the loose connective tissue between the thyroid follicles Often the nuclei of mast cells cannot be seen because of the density of the granules.

Plasma cells. These cells are end-stage lymphocytes that have invaded tissues and are actively secreting antibodies. Plasma cells are usually found in high numbers under epithelia that are exposed to the external environment, such as the skin and the lining of the gastrointestinal tract, where they secrete antibodies to help defend against invading pathogens.
4, esophagus wall. Look deep to the dark-staining epithelium (what type of epithelium is this?), in the loose connective tissue for scattered cells that are relatively small and oval-shaped. Plasma cell nuclei are usually oval, darkly stained and off-centre in the cytoplasm, which is usually stained purple.

C) Fibres. Connective tissue fibres are of 2 main types, made by fibroblasts and some other cell types (such as myocytes). The monomers of which these fibres are made are secreted into the extracellular environment and self-assemble into their respective fibre types. Collagen fibres provide strength and resistance to mechanical deformation of tissues, and elastin fibres provide flexibility.

Collagen. Of the many types of collagen fibres, you will be able to see the most abundant, Type I, in the slides below.
26, 68, skin. Look in the dermis of the skin to find the pink-stained bundles of Type I collagen fibres running in many directions. Some bundles will be sectioned along their length while others will be seen in cross-section. It is these very strong collagen bundles in the dermis that give skin its strength. Why are the bundles oriented in different directions? Leather, made from animal hides, is mostly collagen fibres in the dermis that have been chemically cross-linked together to make a very strong sheet of tissue.

Elastin. Most loose connective tissue throughout the body contains elastin fibres, allowing this tissue to stretch in response to changes in volume or shape of body organs, particularly the skin and hollow organs such as the gut and the lungs.
29, epiglottis. The epiglottis is a very flexible structure lying at the opening of the trachea, acting as a flap valve to prevent swallowed material from entering the airways. The core of the epiglottis is made up of elastic cartilage, containing large quantities of dark-staining elastin fibres (how does form follow function here?). The elastin fibres make an extensive network between the cartilage cells (chondrocytes, the hollow, unstained round spaces in the core of the tissue). The layers around the outside of the cartilage are the covering epithelium and some pink-stained collagen fibre bundles.

D) Types of proper connective tissue. The classification of proper connective tissue is based on the density of fibres (the more fibres, the fewer cells) and the fibre organization. In addition, the degree of vascularity varies in the different types; the denser the connective tissue, the fewer blood vessels are present.

Loose. This type of connective tissue has the lowest fibre density (therefore is the most highly cellular), the highest concentration of ground substance and the most blood vessels of the types considered here. All cell types above are present in this type of tissue.
4, esophagus. Loose connective tissue forms the substrate for the epithelium. See if you can find all connective tissue cell types. Start with the easiest cells to identify (ie the most common) and work through all the types. Blood and lymphatic vessels are evident as hollow structures lined with simple squamous epithelium (named endothelium in vessels) and have relatively thin walls. Collagen fibres are stained pale pink. Elastin, although present, will not be seen because the stain used on this tissue does not show this fibre type.

Dense. Collagen fibres make up a much greater proportion of this type of tissue than in loose connective tissue; look carefully at the orientation of the bundles of collagen fibres to distinguish between dense regular and irregular connective tissue.

68, skin. You have visited this slide several times and now know where to look to see the interwoven collagen bundles of dense irregular connective tissue. Note the variation in orientation of these bundles throughout the tissue. What is the implication of this arrangement for the overall strength of the skin? How many cell types can you identify? Compare the number and locations of fibroblasts and blood vessels in this tissue with the number of these structures in loose connective tissue.

91, tendon. This slide shows an example of dense regular connective tissue. How are the collagen fibres (purple stain) oriented? You will have to look carefully to see fibroblast nuclei. Can you identify any other cell types or blood vessels? Compare the structure of this type of connective tissue with the other types you have examined.

2) Muscle.

Objectives:
1) differentiate the appearance of myocytes of the three types of muscle in the body
2) understand the relationship of connective tissue with muscle tissue
2) identify muscle types as they appear in complex tissues and organs

Striated muscle includes both skeletal muscle (contracts under voluntary nervous control) and cardiac muscle (the heart beat originates from the cardiac pacemaker cells, not the nervous system). Smooth muscle has no striations, and its contractions are under involuntary control by the autonomic nervous system.

Skeletal muscle.
The myocytes (muscle cells, also called muscle fibres) of this muscle type are very long, unbranched, cylindrical cells with multiple nuclei (what is the origin of these nuclei?) which are pushed to the periphery of the cytoplasm because of the dense packing of myofibrils (contractile proteins) inside the cell. Each myocyte is anchored to its neighbors by endomysium, visible in the slides below. Bundles of myocytes, called fascicles, are surrounded by the perimysium, and the whole muscle is covered in a sheath of connective tissue termed epimysium. Collagen fibres within all levels of connective tissue associated with skeletal muscle are connected so that, when myocytes contract, they pull together on the structure to which the muscle is attached.

86 skeletal muscle and tendon. The tendon (made of type I collagen fibres) is stained dark red in this slide. The myocytes are stained a lighter reddish color, and are mostly cut longitudinally or obliquely; you can therefore see multiple nuclei around the periphery of each myocyte along its length. Examine the banding pattern of contractile protein within each myocyte, and review the structural origin of this banding. To maximize the light-dark contrast within the bands under the microscope, you may have to change the lighting of the tissue by adjusting the position of the substage condenser; closing the iris diaphragm may also help to show up the bands. This tissue sample was fixed (a chemical process used to preserve tissues for microscopic study) with the muscle relaxed. How would the banding pattern change if the muscle sample had been taken in a contracted state? (hint: what happens to the A and I bands as myocytes contract?) Between myocytes, look at the endomysium. This contains nerves (not visible in this slide), reticular fibres (Type III collagen associated with the basement membrane, also not visible) and blood vessels, mostly capillaries (which may contain some erythrocytes). Can you see how the tapered ends of myocytes are attached to the tendon? In this region the Type I collagen fibres of the tendon are directly joined to the reticular fibres in the basement membrane surrounding each myocyte.

140 skeletal muscle, longitudinal section. The banding is not very clear on this slide, but you can clearly see multiple thin, flat nuclei within the myocytes. Can you find any small blood vessels in the endomysium?

17 tongue. This section includes some of the epithelium covering the tongue (what kind is it?), dense irregular connective tissue deep to the epithelium, and a large number of fascicles of skeletal muscle that make up the bulk of the tissue in this section. Bundles of collagen fibres are stained a lighter pink than the myocytes, and do not show any internal banding. Muscle fascicles are oriented in multiple layers running in different directions in the tongue, giving this structure the capability to move freely in any direction. A single section of the tongue will thus contain groups of myocytes cut in cross-section, obliquely, or longitudinally. Banding is best seen in longitudinally-sectioned myocytes. The connective tissue surrounding each fascicle is perimysium, and contains collagen fibres, some blood vessels and adipocytes. The vessels of the perimysium are connected to capillaries in the endomysium between individual myocytes within each fascicle. There is no epimysium in the tongue.

Cardiac muscle

This type of muscle is striated but the myocytes are short, may branch, have only one centrally located nucleus per cell, and are joined end-to-end by intercalated disks. These disks contain both mechanical cell-to-cell anchors (adhering junctions) and gap junctions (what is the function of these?).

20, ventricular cardiac muscle. Compare the structure of the myocytes on this slide with that of skeletal muscle myocytes. Try to locate an area on the slide where the majority of myocytes are sectioned longitudinally, in order to clearly see the intercalated disks. These structures are visible as thin dark lines oriented transversely to the long axis of the myocytes and will show up at higher magnification with careful focusing. Banding is not as intense in cardiac myocytes as in skeletal myocytes (why?) so you will have to adjust the microscope condenser (as for slide 86 above) to optimize visualization of the bands. Try to find some regions on the slide where the myocytes are cut in cross-section to confirm that the nuclei tend to be centrally located in the myocytes. Between myocytes is the endomysium; there will be many capillaries here. The general organization of myocytes in the heart is in multiple continuous sheets around the cavity of the ventricular chamber, so there is no perimysium or epimysium as recognized in skeletal muscle.

Smooth muscle

The cells of smooth muscle are spindle-shaped, tapering to the ends from the thicker mid-portion of the cell where the single nucleus is centrally located. The nuclei are elongated ovals, with their long axis in the direction of the long axis of the cell. Smooth muscle is found in the walls of hollow organs such as blood vessels, lung, intestine, bladder and pelvic organs. There is no banding pattern visible in smooth muscle myocytes (why?).

8, ileum. The ileum is part of the small intestine, a tubular organ which has smooth muscle in its wall that alternately contracts and relaxes in each part of the gut to move boluses of digesting food along the length of the tube. For this exercise, focus on the two thick layers of myocytes furthest away from the epithelium. Myocytes in the outermost layer of muscle are oriented parallel to the longitudinal axis of the gut tube while myocytes in the adjacent inner layer are oriented circumferentially around the lumen. You can see that there is a distinct border between these two layers. What is the shape of the myocytes in longitudinal view? Are the nuclei long and straight, or are some bent or curled? If so, what does this imply about the contractile state of the tissue when the sample was taken? Can you see any details in the endomysium? Smooth muscle does not have a perimysium or epimysium. Given the orientation of myocytes in these layers, can you tell how the section on your slide was cut relative to the long axis of the intestine?

4, esophagus. Look for the layers of muscle in the esophageal wall. Compare the orientation of the myocytes in this part of the gastrointestinal system with that in the ileum. Are any of the myocytes striated?