BIOLOGY 211: HUMAN ANATOMY & PHYSIOLOGY

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 **MUSCLE CYTOLOGY AND HISTOLOGY**

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Reference: Saladin, KS: Anatomy and Physiology, The Unity of Form and Function. 8th ed. (2018)

 **Be sure you have read and understand Chapter 11 before beginning this lab.**

**INTRODUCTION:**

 Muscle tissues, which generate a pulling force to produce movement, are distributed throughout the body. They make up a large majority of some organs such as the gastrocnemius, heart, and uterus whose main function is to contract. In other organs such as the intestine or skin, muscle tissue is located only in specific regions. The human body contains three types of muscle tissue:

 - **Smooth muscle tissue** is located in the walls of hollow organs of the digestive, respiratory, circulatory, renal, and reproductive systems. It is also found in some non-hollow organs such as the skin. Contraction of smooth muscle is never under our conscious control; thus it is classified as being ***involuntary****.* Because its cells do not have a banded appearance like the other two types of muscle tissue do, it is also classified as being ***nonstriated***.

 - **Cardiac muscle tissue** is found only in the wall of the heart. It is also classified as being ***involuntary***, but its cells do have a banded appearance so it is also classified as being ***striated***.

 - **Skeletal muscle tissue** forms the large, named muscles which move bones, cartilages, and the skin of the face. It is classified as ***voluntary*** because its contraction is under conscious control of the nervous system, and as ***striated*** because its cells have the same banding appearance as those in cardiac muscle.

 In a previous exercise, you learned to recognize each of these three types of muscle tissue under the microscope. In this exercise, we will go beyond that to learn more about both the cells of muscle tissues, called **myocytes**, and the tissues themselves.

**SKELETAL MYOCYTES**

 The *organs* we call skeletal muscles, such as the biceps brachii or gastrocnemius, generate force by the contraction of the individual myocytes which form the skeletal muscle *tissue*. If we look more closely within these cells, we see that this contraction is actually caused by interactions between **thin myofilaments** and **thick myofilaments**, which form structures called **myofibrils**.

 Myocytes of skeletal muscles are among the largest cells in the human body. They range from 10 μm to 100 μm in diameter and 100 μm to 30 cm in length, being larger (both wider and longer) if they produce more force and smaller when they need to produce less force and finer movement. Skeletal myocytes appear as very long cylinders which do not branch, leading earlier anatomists to incorrectly call them “fibers” rather than cells. Approximately one third of a myocyte’s total volume consists of **myofibrils**, which generate the force of contraction, and another third of its volume consists of the **mitochondria** necessary to provide the energy for this. A mature myocyte contains hundreds of fully functional **nuclei** which are always located out at the edge of the cylindrical cell, never deep within it.

**1**: Examine the model which shows a cross-section of part of a myocyte. With the assistance of Figure 11.2 in your Saladin text, identify its:

 **Sarcolemma** (notice how it forms folds under the myoneural junction)

 **Myofilaments** forming **Myofibrils** with **A-bands, I-bands,** and **Z-lines**

 **Sarcoplasmic reticulum** surrounding the myofibrils

 **Transverse tubules** forming **triads** with the terminal cisternae of the

 sarcoplasmic reticulum

 **Mitochondria** in between the myofibrils and deep to the myoneural junction

 One **Nucleus** (purple) at the periphery of the myocyte

 **Myoneural** or **Neuromuscular junction** with an extensively branching neuron

 **Endomysium** (partially shown) of loose areolar connective tissue

Notice that the myofibrils are all oriented in the same direction - parallel to the long axis of the myocyte - and that their A-bands and I-bands line up across the entire width of the cell.

**2**: In the space below, draw from memory the arrangement of thin and thick myofilaments as they would form three consecutive **sarcomeres** of the same myofibril. Use dark or thick lines to show **thick myofilaments** and lighter or thinner lines to show **thin myofilaments**. On your drawing, label the (1) **A-bands**, (2) **I-bands**, and (3) **H-zones**. Be sure to include (4) **Z-lines** (also called Z-discs) at the proper places. Figures 11.2, 11.3, and 11.5 in your Saladin text will help you do this if necessary, but your drawing should look much more simple than any of those. The purpose is not to produce a pretty picture, it’s to see if your brain knows the structure of a myofibril, so you should be able to do this with your book closed.

Compare the drawings of all members of your lab group. Be sure everyone understands how thin myofilaments and thick myofilaments interact to form sarcomeres and myofibrils.

**3**. Explain to others in your lab group how molecules of the protein myosin are arranged in a thick myofilament. Explain how the proteins actin, troponin, and tropomyosin are arranged to form a thin filament. Do not move on until everyone in your group understands this.

**4**: Examine Figure 11.2 of your Saladin text and identify the network of small tubules which form the **sarcoplasmic reticulum** (“network”) within a myocyte. Also identify the **transverse tubules**, which are actually inward extensions of the sarcolemma and run perpendicular to it, deep into the cell. Notice that the sarcoplasmic reticulum surrounds each myofibril. While it doesn’t show as well, you should realize that the transverse tubules also branch and surround the myofibrils. Note that each transverse tubule runs between two large tubes (the **terminal cisternae**) of the sarcoplasmic reticulum to form a triad.

**SKELETAL MUSCLE TISSUE AND ORGANS**

While myocytes are certainly the main part of skeletal muscles (organs) such as your deltoid or gluteus maximus muscles, they are not the only component. These organs also include layers of connective tissue. As myocytes pull on these, they transmit their force to the bone (or other structure) that the muscle is trying to move. These connective tissues also contain the blood vessels and the nerves which are going to the myocytes. At either end of the muscle, the myocytes disappear and these connective tissues remain to form the tendon.

**5**: Examine Figure 10.1a of your Saladin text and be sure you understand how the following tissues and structures are organized to form a skeletal muscle (organ).

 - **Myocytes**

 - **Endomysium**

 - **Fascicles**

 - **Perimysium**

 - **Epimysium**

 - **Tendons**

The model of the myocyte which you examined in #1 above shows the **endomysium**. Be sure you can identify this. Do not confuse the endomysium (a thin layer of rougher connective tissue) with the sarcolemma (the smooth plasma membrane of the cell) which is deep to it.

**6**: Starting with the myocyte, list (in the proper order) the connective tissue layers which transmit the force of contraction from myocytes to tendons:

 Myocytes pull on \_\_\_\_\_\_\_\_\_mysium,

 which pulls on \_\_\_\_\_\_\_\_\_mysium,

 which pulls on \_\_\_\_\_\_\_\_\_mysium,

 which pulls on the tendon.

These connective tissue parts of skeletal muscles (organs) are able to transmit the force of

contraction from myocytes to tendons without stretching because they contain of what specific

type of fiber? (see Figure 5.16 in your Saladin text if necessary).

**7**: Close your book. **From memory,** draw the arrangement of **myocytes**, **fascicles**, **endomysium**, **perimysium**, and **epimysium** as they would appear in a muscle cut in cross-section. Figure 10.1a of your Saladin text shows these in three-dimensional view, but your drawing should not replicate this. Keep it simple and be sure each of these components is easily identifiable. Remember: your objective is not to get a nice, pretty picture, but to be sure you know where all of these components are located relative to the others.

**8**. With a microscope, examine under low power slide #15. You may have to move the slide around a bit, but you should observe that it includes skeletal muscle tissue which has been cut in both longitudinal section and cross section.

 Under medium and high powers, examine the tissue cut longitudinally. By moving the fine focus up and down gently you should observe the banded or striated appearance, similar to Figure 10.1(c) or 11.1 in your Saladin text. Note that any nuclei which are in sharp focus are at the edges of the myocytes. Some nuclei which are out of focus may appear to be deeper in the cell. These are indeed still on the periphery of these cylindrical cells, above or below the rest of the cell. Identify the thin layer of connective tissue between myocytes.

What is this layer of connective tissue called? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

 Under medium and high powers, examine the tissue cut in cross section. Be sure you understand why you do not see banding or striations in this orientation. Identify the myocytes, noting that nuclei are again all found at the edges of the cells. Identify the thin layer of connective tissue (called \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_) which surrounds each cell.

 Examine the cross section of skeletal muscle under low power. Note that the myocytes are grouped into bundles, or **fascicles**, separated by a thicker layer of connective tissue.

What is this layer of connective tissue called? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**9.** Skeletal muscle tissue is considered *voluntary* because its myocytes contract only when stimulated by a nerve cell, or *neuron*. If this neuron is damaged, or if its communication with myocytes is interrupted, those myocytes are unable to contract.

 Examine Figures 11.6, 11.7, and 11.8 of your Saladin text and identify on these diagrams the **neuromuscular** or **myoneural junction** where the end of a nerve cell communicates with the sarcolemma of a myocyte. One of these is also shown on the model you examined in #1 above.

Notice:

 a) A single nerve fiber (actually, it is called an “**axon**” of a neuron) divides to send branches to

 many different myocytes. As each branch approaches a myocyte it divides into many

 smaller branches, each of which ends in a swelling called the **synaptic knob** or **axon**

 **terminal** or **terminal bouton.**

 b) The synaptic knob of the nerve cell never actually touches the muscle cell (myocyte).

 They are always separated by a thin space called the **synaptic cleft**.

 c) The end of the nerve cell contains many small vesicles (“**synaptic vesicles**”) containing

 the neurotransmitter chemical **acetylcholine**.

 d) The sarcolemma of the myocyte forms a depression deep to the axon terminal of the nerve

 cell, and it is folded (“**junctional folds**”) within this area. This increases its total surface

 area to which acetylcholine can bind when the neruron stimulates the myocyte to contract.

**10**. With a microscope, examine slide #39 from the set of slides in your lab drawer. Identify the myoneural junction on this slide.

 - What is a “motor unit”?

 - Why would a smaller motor unit allow more “fine control” of muscle contraction?

**CARDIAC MYOCYTES and CARDIAC MUSCLE TISSUE**

 The heart is the only organ in the human body which contains **cardiac myocytes** and therefore **cardiac muscle tissue**. Although it is *involuntary*, meaning that these myocytes spontaneously generate their own signal to contract even if they are not connected to nerve cells, the cytology of cardiac myocytes is very similar to that of skeletal myocytes.

 a) Both skeletal myocytes and cardiac myocytes have **thin myofilaments** and **thick**

 **myofilaments** which are organized into **myofibrils**.

 b) These myofibrils of cardiac myocytes have the same banding pattern as those of skeletal

 myocytes, including **A-bands**, **I-bands**, **H-zones**, and **Z-lines**, and make up approximately

 one-third of the total volume of the cell.

 c) Approximately one third of the total volume of a cardiac myocyte consists of **mitochondria,**

 which provide the energy for contraction.

 d) The myocyte contains **transverse tubules**, which are inward extensions of the sarcolemma,

 and a network of **endoplasmic reticulum** which surrounds the myofibrils.

There are, however, a few differences between cardiac myocytes and skeletal myocytes

 e) Cardiac myocytes are much smaller: 50 to 100 μm in length and 10 to 20 μm in diameter.

 How does that compare with the size of skeletal myocytes?

 f) Although still cylindrical in shape, cardiac myocytes branch at one or both ends – see

 Figures 5.26 and 19.11 in your Saladin text.

 g) Each cardiac myocyte contains only one, or occasionally two, nuclei, located in the center of

 the cell rather than its periphery (Figures 5.26 and 19.11).

 h) The endoplasmic reticulum does not form terminal cisternae in cardiac myocytes, so there

 are no triads.

 There is also a feature unique to cardiac muscle tissue which almost always allows you to differentiate it from skeletal muscle tissue if you look for it. Cardiac myocytes are held together very tightly end-to-end by **desmosomes** and are electrically “coupled” by many **gap junctions** which allow ions flow between cells. These adhesive and electrical junctions form an **intercalated disk** between the ends of the two cells, and this can be identified as a relatively dark line when you examine cardiac muscle with a microscope.

**11**. In Figures 5.26 and 19.11 (actually, they are the same photograph) of your Saladin text, identify the intercalated disks between adjacent cardiac myocytes. Note that a disk is found only where two cells meet end-to-end, not along the sides of the cells, so it lies perpendicular to the long axis of the myocyte.

**12**. Examine slide #1 under the lowest power of your microscope. As you scan around the tissue, you will notice that the cells change orientation from one region to another. Select a region of the tissue in which the myocytes have been cut in longitudinal section (similar to Figures 5.26 or 19.11 in your Saladin text) and examine this region with the medium and high powers of the microscope. By focussing up and down gently, as you did earlier for skeletal muscle tissue, you should see the banding pattern, although it is not as distinct as it is in skeletal muscle. Look for intercalated disks where cells meet end-to-end. These may be difficult to find at first, but once your eyes (and brain) get used to looking for them you will see them all over this slide.

**SMOOTH MYOCYTES and SMOOTH MUSCLE TISSUE**

 Smooth muscle tissue is part of many different organs in many different systems of the human body. Most often it is found in hollow organs which change their size or shape. It is *involuntary*, meaning that these myocytes contract even if they are not connected to nerve cells, and unlike either skeletal muscle or cardiac muscle it is *nonstriated*.

 Smooth muscle myocytes are not cylindrical in shape like either cardiac myocytes or skeletal myocytes. They are widest in the middle of the cell, where the single nucleus is located, but then they taper to narrow points at both ends - resembling the “spindles” used by weavers and tailors about a hundred years ago. Although they are about the same length as cardiac myocytes, 50 to 100 μm, they are significantly narrower in cross-section, rarely more than 8 to 10 μm in diameter.

**13.** Examine Figures 11.20, 11.22, and 11.23 in your Saladin textbook. Identify this “spindle” shape (wide in the middle, tapering at the ends) for individual cells and how this allows them to fit together in a very tight pattern with very little room for extracellular matrix. Also note the location of the nuclei in the centers of the cells.

**14.** Examine the model of smooth muscle. Here again, note the spindle shapes of the cells and how this allows them to pack tightly together. This model also shows a capillary (the smallest of the blood vessels in the human body) - compare the diameters of the myocytes with the diameter of this capillary.

**15.** In your Saladin text, examine Figures 11.20; 25.2; 25.12; 25.13 and 28.3 which diagram the location of smooth muscle tissue in various organs. It is typically arranged in patterns which allow it to both shorten and narrow the diameter of an organ.

**16**. Examine the following slides and identify the smooth muscle in each:

 #5 - small intestine of a frog

 #19 - oviduct (Fallopian tube)

 #24 - stomach

 #26 - duodenum

 #33 - urethra

 #35 - ureter

**17.** Smooth muscle is also located in the iris (colored part) of your eye and can change the size of the pupil to adjust to differing amounts of light.

 Have your lab partner sit facing you in a relatively dark part of the room for two or three minutes. While watching her or his iris (things will change quickly), shine a bright light into one eye. You should see the iris quickly get smaller as smooth muscle cells which surround it contract.

**18.** One last review before you leave the lab: Explain to the other members of your lab group how skeletal muscle, cardiac muscle, and smooth muscle are SIMILAR to each other. Explain how these three tissues are DIFFERENT than each other. Explain how these similarities and differences affect their functions in different organs of the body.