

LEARNING OBJECTIVES OUTLINE

I. MUSCLE TISSUE

A. Muscle cells (myocytes)

B. Extracellular matrix

II. CLASSIFICATION OF MUSCLE: TYPES

A. Striated: Skeletal

1. *Typical skeletal muscle*: attached to bone; move axial and appendicular skeleton
2. *Visceral striated (skeletal) muscle*: not attached to bone
 - a. Intrinsic tongue muscles
 - b. Intrinsic pharyngeal muscles
 - c. Muscularis externa of upper esophagus
 - d. Lumbar portion of diaphragm

B. Striated: Cardiac

C. Smooth

D. Other Contractile Cells

1. Myoepithelium
2. Myofibroblasts
3. Perineurial cells
4. Testicular myoid cells

III. STRIATED MUSCLE

A. General features and properties

1. Vertical striations (\perp to long axis of cells)
2. Arrangement of striations
 - a. Alternating A and I bands
 - i. A (anisotropic) bands: dark
 - ii. I (isotropic) bands: light
 - b. Z lines bisect I bands
 - c. H bands
 - d. M lines
 - e. Sarcomeres: Z line to Z line
3. Principal muscle filamentous proteins
 - a. Myosin (thick filaments)
 - i. Molecular structure
 - ii. Motor protein
 - b. Actin (thin filaments)
 - i. Molecular structure
 - ii. Myosin interaction
4. Organization of thick and thin filaments
 - a. Myofibrils

b. Sarcomeres

B. Skeletal muscle

1. Organization
 - a. Anatomic muscles (named)
 - b. Fascicles
 - i. Bundles of muscle cells
 - ii. Surrounded by CT (perimysium)
 - c. Myofibers (=muscle cells)
 - i. Length: several mm - one meter (sartorius)
 - ii. Diameter: 10-100 μm
 - iii. Multinucleate syncytial cells
 - d. Myofibrils
 - i. Basic subunit of myofiber
 - ii. Intracellular bundles of myofilaments surrounded by SR, mitochondria, etc
 - iii. Myofibril = linear array of n sarcomeres
 - e. Myofilaments
 - i. Thick (myosin) filaments
 - ii. Thin (actin) filaments: G and F actin
 - iii. Troponin-tropomyosin system
 - iv. Associated proteins: titin, myomesin, nebulin, etc.
2. Cell types
 - a. Myoblasts
 - b. Myocytes (=myofibers): syncytium
 - c. Satellite cells
 - i. Myogenic stem cells
 - ii. Inside external lamina
 - d. Other stem cells
3. Cellular components of myocytes
 - a. Sarcolemma (=plasmalemma)
 - b. Sarcoplasmic reticulum (SR) (=smooth ER)
 - c. Triads (located at A-I junctions)
 - i. Transverse (T) tubules (1)
 - ii. Terminal cisternae of SR (2)
 - iii. Excitation-contraction coupling
 - d. Sarcomere: basic unit of muscle contraction
 - i. Actin and myosin filaments
 - ii. Boundary: Z-line to Z-line
 - iii. Bands: A, H, I, M, Z

- e. Costameres
 - i. Cytoskeletal lattice that links sarcolemma with adjacent sarcomeres at I bands
 - ii. Vinculin and other proteins
 - f. Myoglobin
 - 4. Inter/Extracellular components
 - a. External lamina
 - b. ECM proteins (e.g., tendon attachment)
 - c. Neuromuscular junction
 - i. Motor end plate
 - ii. Synapse: motor nerve-muscle cell
 - d. Motor unit
 - i. Single motor neuron +
 - ii. Group of muscle fibers
 - 5. CT Sheaths
 - a. Endomysium (myofiber)
 - b. Perimysium (fascicle)
 - c. Epimysium (muscle)
- B. Cardiac muscle**
1. Characteristics
 - a. Branched myofibers
 - b. Cardiocytes arranged end-to-end
 - i. Intercalated disks
 - ii. Cardiocytes attached to two or more adjacent cells
 - c. One or two nuclei per cardiocyte
 2. Types of cardiocytes
 - a. Cardiac myocytes: contractile
 - b. Cardiac myocytes: conducting (cardiac conduction system)
 - i. Narrow diameter fibers
 - ii. Wide (=Purkinje fibers)
 - c. Cardiac myocytes: endocrine
 - i. Specialized contractile cells
 - ii. Secrete natriuretic peptide hormones (ANP, BNP): natriuretic, diuretic, and vaso-relaxant activities
 3. Cellular components
 - a. Similar to skeletal muscle but with some differences
 - i. Incomplete myofibrils
 - ii. "Diads" (located at Z line) consist of T tubules (wider than those in skeletal muscle) and single profiles of SR (no terminal cisternae)
 - b. Costameres
 4. Inter/Extracellular components
 - a. Intercalated disks
 - i. Transverse portion: fascia adherens (α -actinin, vinculin); desmosomes
 - ii. Longitudinal (lateral) portion:

- gap junctions; desmosomes
- b. External lamina

IV. SMOOTH (PLAIN) MUSCLE

A. Characteristics of myocytes

1. Generally unbranched; some branching (e.g., blood vessels, urinary bladder)
2. Length: 15-500 μ m
3. Diameter: 3-8 μ m

B. Cellular components

1. Caveolae ("monads"): no T tubules
2. Cytoplasmic dense bodies
 - a. α -actinin
 - b. Z-line equivalents
3. Cytoplasmic filaments
 - a. Contractile: actin & myosin
 - b. Intermediate: desmin; desmin & vimentin (vascular smooth muscle)

C. Inter/Extracellular components

1. Gap junctions (variable)
2. External lamina
 - a. Collagen IV
 - b. Laminin
3. Argyrophilic reticulum
 - a. Collagen III
 - b. Elastic components
 - c. Proteoglycans

OVERVIEW

Muscle is a tissue characterized by **irritability** and **contractility**. It is composed of elongated cells called **myocytes** that contain contractile proteins organized as cytoplasmic filaments. Muscle cells are attached to bones and soft tissues, and thereby accomplish mechanical movements as a consequence of their contraction (shortening). Traditionally, three types of muscle tissue are recognized: **skeletal**, **cardiac**, and **smooth**. Skeletal and cardiac muscle are classified as **striated** muscles based on the appearance of striations that run perpendicular to the long axis of the cells. The striations are due to a highly ordered, repetitive organization of two filamentous contractile proteins - *actin* and *myosin*, which are arranged into *thin* and *thick filaments*, respectively. Smooth muscle lacks striations due to a less structured, looser arrangement of its contractile filaments. In muscle terminology, the **sarcolemma** refers to the muscle cell membrane, the **sarcoplasm** refers to the cytoplasm, and the **sarcoplasmic reticulum** refers to the smooth endoplasmic reticulum.

SKELETAL MUSCLE

Skeletal muscle cells are long cylindrical myocytes that are referred to as **fibers** or **myofibers**. Each fiber is surrounded by an **external (=basal) lamina**.

1. Origin and syncytial development.

Developmentally, myogenic stem cells give rise to uninucleate **myoblasts**, which fuse to form multinucleate skeletal muscle cells (myofibers). Thus, the adult phenotype of skeletal muscle cells is a true morphologic **syncytium** [Gr. *syn* =with, together + *cyt-* =cell].

2. Size. Myocyte length varies widely from a few mm to a meter or more in the sartorius [L. *sartor* =tailor] muscle, which extends from the anterior superior spine of the ilium to the medial border of tuberosity of the tibia. The diameter ranges from 10-100 μm .

3. Nuclei. Due to their syncytial development, skeletal myocytes are polyploid cells, which contain multiple ovoid nuclei. In mammals, the nuclei are typically located in the peripheral sarcoplasm just beneath the sarcolemma. This characteristic distinguishes skeletal muscle cells from cardiac muscle cells (see below), which usually have one centrally-located nucleus.

4. Light microscopic appearance. In skeletal muscle cells stained with H&E, the sarcoplasm appears homogeneous or stippled (due to myofilaments) in cross section and striated in longitudinal section.

a. Striations. Composed of alternating **dark** and **light bands**, the repetitive striations seen in myocytes result from the vertical summation of smaller bands that occur in **myofibrils**. Myofibrils are the smallest structural and functional subunits of myocytes seen at the LM level. They are arranged in register within the sarcoplasm, parallel to the long axis of the muscle fiber.

i. Dark bands are called **A bands** because they are **anisotropic** (birefringent) in polarized light (i.e., they rotate plane polarized light). The parallel array of thick filaments are mainly responsible for the LM appearance of the A band. In the center of this band a paler region, called the **H band** [formerly *Hensen disk*], can be seen in relaxed muscle. It represents an area of thick filaments that are not overlapped by thin filaments (see below). At the middle of the A band (and the H band) is a middle line called the **M line**.

ii. Light bands are called **I bands** because they are **isotropic** in polarized light (i.e., they do not rotate plane polarized light). A dark transverse line, called the **Z line** (Z disk) [Ger. *zwischen* =between + *scheibe* =disks], bisects each I band.

b. Sarcomeres are defined as the basic units of contraction in striated muscles. A single sarcomere extends from one Z line to the next and contains one A band separating two semi-I bands. Thus, myofibrils are composed of a series of tandemly-arranged sarcomeres consisting of interdigitating polarized thin filaments (*plus end toward Z line; minus end toward A band*) and bipolar thick filaments (*myosin heads toward each semi-I band*).

5. Electron microscopic appearance. In electron micrographs, the repeating pattern of bands and sarcomeres is due to the arrangement of the myofilaments. Myofilaments represent the thick myosin filaments and the thin actin filaments. These structures are not resolved at the LM level.

a. Thin filaments are composed of several proteins, but primarily of actin and two important regulatory proteins called **tropomyosin** and **troponin**.

i. Actin is a long fibrous structure (**F-actin**), which is composed of two strands of spherical or globular **G-actin** monomers that are twisted in a double helix. The filament is polar and contains **myosin-binding sites** on the G-actin monomers.

ii. Tropomyosin consists of two polypeptide chains in a form that is referred to a coiled-coil, alpha-helix. Tropomyosin molecules are arranged end-to-end (each spans 7 G-actin units) to form filaments, which lie in the grooves of the actin helix.

iii. Troponin is composed of three polypeptides: **TnT** binds to tropomyosin at intervals along the thin filament; **TnC** binds calcium ions; and **TnI** inhibits actin-myosin interaction.

b. Thick filaments are composed primarily of composites of the protein myosin II.

i. Myosin II is a polarized molecule that contains **two** heads and a tail. The heads are globular regions that contain molecular motors that utilize the thin actin filaments as tracks.

ii. Orientation of myosin II. In thick filaments, many polarized myosin molecules are arranged with their heads oriented toward the ends of each filament and the tail portions associated

toward the center. Thus, a **bare zone** that lacks heads and contains only tails occurs in the central region of each thick filament.

iii. Myosin heads function as active sites for adenosine triphosphatase (ATPase) activity and as sites that bind to actin.

c. Arrangement of filaments in sarcomeres. Thick filaments occupy central portions of the sarcomere; thin filaments attach at each end to the Z lines and run parallel to, and between, the thick filaments.

i. I bands are composed of thin filaments. Each sarcomere has $\frac{1}{2}$ of an I band at its ends. Thus, a whole I band is shared between adjacent sarcomeres.

ii. A bands are composed mostly of thick filaments and the thin filaments between them.

iii. H bands are composed of only thick filaments, and in relaxed muscle represent the area between the ends of thin filaments that are attached to each Z line at the other ends. In contracted muscle, when the thin filaments merge or overlap, the H band disappears.

iv. Actin and myosin together represent approximately 55% of the total proteins in striated muscle.

6. Costameres. Vinculin [L. *vinculum* = bond], an actin-binding protein that occurs in epithelial cells, fibroblasts, and myocytes, has been found to be involved in the attachment of actin filaments to integral proteins in the plasma membrane (e.g., Geiger, B. *Proc Nat Acad Sci USA* 77:4127-4131, 1980; Tokuyasu, KT, *Proc Nat Acad Sci USA* 78:7619-7623, 1981).

a. Structure. Using an antibody to vinculin, immunohistochemistry revealed an orthogonal (mutually-perpendicular) cortical lattice-like structure associated with the inside of the sarcolemma in skeletal muscle cells; the transverse components of this lattice, which couple the sarcolemma to the I bands of immediately adjacent myofibrils, were named **costameres** [L. *costa* = rib + Gr. *meros*, part](Pardo, JV. *Proc Nat Acad Sci USA* 80:1008-1012, 1983). Although costameres occurred along the length of I bands of peripheral sarcomeres, they were found to be bipartite due to a discontinuity at the Z lines. Important integral proteins of the sarcolemma that are concentrated at the level of costameres are integrins (e.g., Belkin, AM et al. *J Cell Biol* 132:211-226, 1996).

b. Function. Costameres mechanically integrate muscle contractile units with the plasma

membrane and in turn to the ECM in the lateral dimension (e.g., Danowski, BA et al. *J Cell Biol* 118:1411-1420, 1992). In addition, they appear to maintain the spatial organization of the contractile apparatus by preventing the sarcolemma from being markedly displaced from adjacent cytoplasmic components during contraction when extensive sarcomere shortening occurs.

Muscle Contraction

1. Sliding filament mechanism of muscle contraction has been accepted as the explanation of how a muscle cell shortens on contraction.

a. Filament length is constant. In the shortening of muscles during contraction, myofilaments maintain constant lengths because thin filaments slide past thick filaments.

b. Movement of myofilaments. The sliding movement is due to cross-bridges formed between the myosin heads (motor proteins) and binding sites on the actin monomers.

i. Myosin motors, which connect the thick and thin filaments during muscle contraction, move along the actin tracks in a ratcheting fashion until the sarcomere is shortened.

ii. Myosin filaments are fixed. In each sarcomere, the thick filaments remain stationary. However, the ratcheting movements of their myosin heads cause the thin filaments to slide past the thick filaments toward the center of the sarcomere.

c. Tension development is proportional to the number of myosin heads overlapped by thin filaments.

2. Actin-myosin interaction. At the onset of muscle contraction, myosin heads move out from the thick filament backbone to interact with ATP and specific binding sites on actin molecules.

a. Bipolarity of thick filaments. The myosin heads at each end of the bipolar thick filament pulls thin filaments deeply into the sarcomere. As the filaments slide, the sarcomeres and myofibrils shorten. The myosin heads then detach from the actin molecules and the cycle is repeated.

b. Contractile appearance. As a consequence of the actin-myosin interaction, which causes thin filaments to slide into the A band, the following results are observed (*important to know*).

- i. Sarcomeres shorten.
- ii. Z lines are pulled closer together.
- iii. H bands narrow and disappear.
- iv. I bands shorten.
- v. A bands remain the same.
- vi. Myofibrils, and muscles, shorten.

3. Adenosine triphosphate (ATP) provides the energy for muscle contraction in the following manner. ATP-bound myosin is active and binds actin. This complex is altered when ATP is split by ATPase to liberate energy.

4. Transverse (T) tubule system consists of tubules formed by finger-like invaginations of the sarcolemma deep into the muscle cell cytoplasm. The interior of T tubules are continuous with the extracellular space. T tubules are arranged transversely to the myofibrils and deliver waves of depolarization deeply into myocytes.

a. Triads, which are characteristic of skeletal muscle only, are defined as a centrally-located T tubule between two dilated portions of adjacent SR called **terminal cisternae**. There are two triads per sarcomere, and in mammalian skeletal muscle they are located at the junctions between the A and I bands.

b. Excitation-contraction coupling, where depolarization of T tubules is coupled to myocyte contraction, occurs at the level of the triads.

5. Sequence of events. The mechanism of muscle contraction is summarized as follows.

a. Innervation. Skeletal muscle cells are innervated by motor neurons whose cell bodies are located in the central nervous system.

i. Motor unit. The axon of the motor neuron branches in the muscle to contact few to many muscle cells forming a **motor unit**. Motor units with fewer muscle cells are characterized by finer motor control.

ii. Motor end plate. The terminus of each branch forms a specialization at the muscle surface called a **motor end plate**.

iii. Neuromuscular junction. An action potential from the motor neuron arrives at the motor end plate and activates a specialized synapse structure called the **neuromuscular junction**. Release of the neurotransmitter **acetylcholine** from this junction binds to specific receptors on the surface of the adjacent muscle membrane, which causes depolarization of the sarcolemma.

b. Muscle cell response. Electrical impulses (depolarizations) travel along the sarcolemma and deep into the cell via the T tubules, which are extensional invaginations of the sarcolemma.

i. T-tubule currents (depolarizations) stimulate adjacent profiles of sarcoplasmic reticulum at triads (**excitation-contraction coupling**) to release calcium ions (Ca^{++}).

ii. Exposure of myosin binding sites. Released Ca^{++} binds to the TnC unit of troponin and induces movement of tropomyosin so that the myosin binding sites on actin are now exposed.

iii. Contraction. As a result of tropomyosin displacement, the myosin heads bind to actin filaments, which initiates the motor movement and sarcomere shortening.

Muscle relaxation

1. Calcium uptake. The sarcoplasmic reticulum possesses an ATP-driven calcium pumping mechanism, which accumulates calcium within its membrane system and thus reduces the cytoplasmic concentration of calcium around the myofilaments.

2. Actin-myosin inhibition. At low calcium concentrations, TnC is unoccupied and tropomyosin repositions itself so that it sterically blocks the myosin-binding site on each actin molecule.

Connective tissue components

1. Investments. Connective tissue not only binds muscles and muscle cells together but also assists in the generation of force and movement during contraction.

a. Epimysium is the connective tissue sheath that surrounds an entire muscle.

b. Perimysium is the connective tissue septum that surrounds groups (**fascicles**) of muscle cells. These septa are inward extensions of the epimysium.

c. Endomysium is the connective tissue framework, composed mainly of reticular fibers, that surrounds and supports individual muscle cells.

2. Myotendinous junctions. Connective tissue fibers from the endomysium, perimysium, and epimysium come together at the ends of muscles to form fibrous CT called **tendons**. Tendons are

defined as attaching muscles to bones or other structures.

3. Microcirculation. Skeletal muscle is a highly vascular tissue; capillaries are located in the connective tissue surrounding all muscle cells.

Regeneration and repair.

1. Satellite cells are a quiescent population of mononucleate cells that are closely associated with skeletal myocytes (Katz, FRS. *Philos Trans R Soc London Ser B*: 243:221-225, 1961; Mauro, A. *J Biophys Biochem Cytol* 9:493-498, 1961).

a. Location. In adult muscle, satellite cells are found within the external lamina adjacent to the sarcolemma at various locations along myocytes.

b. Function. These cells have been viewed until recently as the major (perhaps only) source of **myogenic stem cells** responsible for postnatal growth, maintenance, regeneration and repair following injury (e.g., Schultz, E. *Med Sci Sports Exercise* 21:S181-S186, 1989). When activated due to injury or trauma, satellite cells enter mitosis, give rise to myoblasts as well as other reserve satellite cells, and fuse with existing myocytes or themselves to effect regeneration and repair of the damaged muscle. This regenerative capacity appears to be limited and decreases with age (e.g., Grounds, MD. *Ann NY Acad Sci* 854:78-91, 1998).

2. Other stem cells. One or more novel stem cell populations [e.g., **side population (SP) cells**, which have characteristics of hematopoietic stem cells] have recently been described to be present in adult muscle and to participate in regeneration of myocytes and satellite cells.

a. Evidence. Recent studies, which have questioned whether satellite cells are the only cells that are important in skeletal muscle regeneration and repair, are summarized in the following reviews (Seale, P & Rudnicki, MA. *Dev Biol* 218:115-124, 2000; Hawke, TJ & Garry, DJ. *J Appl Physiol* 91:534-551, 2001; Zammit, PS & Beauchamp, JR. *Differentiation* 68:193-204, 2001; Goldring, K et al. *J Pathol* 197:457-467, 2002; Askara, A. *Trends Cardiovasc Med* 13:123-128, 2003; Chen, JCJ & Goldhamer, DJ. *Reprod Biol Endocrinol* 1:101-107, 2003; Huard, J et al. *Birth Defects Res Part C*: 69:230-237, 2003; Morgan, JE & Partridge, TA. *Int J Biochem Cell Biol* 35:1151-1156, 2003).

b. Origins. Whether these novel populations of myogenic cells are associated with myocytes, associated CT (undifferentiated mesenchymal cells), blood vessels (pericytes), or migrate from bone marrow remains to be

determined.

CARDIAC MUSCLE

Cardiac muscle cells. Like skeletal muscle cells, cardiocytes are elongated cells that contain sarcomeres, exhibit transverse banding patterns, and are surrounded by an external lamina. The molecular basis for cardiocyte contraction is also viewed to be the same. However, these two types of striated muscle differ in several important ways.

1. Size. Cardiac cells are smaller than skeletal myocytes. They are about 100-150 μm in length and range from 10-35 μm in diameter.

2. Nucleus. Cardiocytes usually possess one centrally-placed nucleus. However, it has been demonstrated that the distribution of mononucleate, binucleate, trinucleate, and tetranucleate human cardiocytes is 74%, 25.5%, 0.4%, and 0.1%, respectively (Olivetti, G et al. *J Mol Cell Cardiol* 28:1463-1477, 1996). Percentages did not change with age (26-93 yrs), myocardial hypertrophy, or ischemic cardiomyopathy.

3. Arrangement: branching myofibers. Unlike skeletal myocytes, cardiocytes are arranged end-to-end so that a **cardiac muscle fiber** is actually composed of numerous cells. Cardiocytes also have branching ends so that they usually contact more than two adjacent cells. Thus, cardiac myofibers are branched.

4. Intercalated disks (IDs) are extensive junctional structures that occur between cardiac myocytes where they are attached to each other at their ends. Because cardiac muscle fibers are composed of individual myocytes that are arranged in tandem, IDs are crucial for cardiac muscle function by providing areas for 1) secure attachment and 2) intercellular communication. In well stained preparations, IDs can be clearly seen between cells at the LM level. However, their constituent parts can only be resolved at the EM level. Close inspection shows that IDs are arranged as step-like structures that follow the zigzag contours that occur at the ends of cardiocytes. They consist of the following components.

a. Transverse portion, which occurs between the ends of tandem cells, contains specialized junctions for attachment: **fascia adherens** and **desmosomes**. Since actin filaments attach to transverse portions of IDs at the

ends of the cells, they serve the function of Z lines.

b. Longitudinal (lateral) portion, which occurs between lateral cells, contains **gap junctions** and desmosomes. Unlike skeletal muscle, cardiac muscle cells are electrically coupled to each other through gap junctions, which is important for cardiac myocyte rhythmicity.

5. Costameres, which appear to be an important cellular specialization for lateral attachment of myofibrils to adjacent sarcolemmal membranes in skeletal muscle, have also been described in cardiac muscle (Pardo, JV et al. *J Cell Biol* 97:1081-1088, 1983). They encircle cardiac myocytes perpendicular to the long axis of the cell and are localized at the I bands of adjacent sarcomeres. However, unlike their discontinuity at the Z lines in skeletal muscle (see above), they are apparently associated with Z lines in cardiac muscle. Perhaps this is related to the fact that T tubules, which are specializations of the sarcolemma, occur at the Z lines in cardiac muscle, but not in skeletal muscle.

6. Cardiac muscle myofibrils are different from those found in skeletal muscle. Due to an important role of extracellular calcium in cardiac muscle contraction, the SR is not as extensive and well developed in cardiocytes as in skeletal muscle myocytes. Thus, cardiac myofilaments are not completely segregated into discrete myofibrils by the SR as in skeletal muscle. Rather, cardiac myofibrils occur as continuous fields that are only partially divided by discontinuous profiles of SR and other portions of sarcoplasm. It should be noted that this distinction is only visible at the EM level. At the LM level, typical myofibrils appear to occur. Finally, unlike skeletal myofibrils, cardiocyte myofibrils vary in length due to the fact that each cell has a variable branching morphology.

7. Types of cardiocytes. Based upon both structural and functional characteristics, three types of cardiac myocyte are identified.

a. Contractile cardiocytes, also referred to as “working myocytes”, are the typical cells of the myocardium that tirelessly contract about 3 billion times during an average human lifespan.

b. Conductile cardiocytes are the cells that make up the fibers of the **cardiac conduction system** (see below). They are specialized for conducting the depolarization currents rather than for contraction. In this respect they resemble nerve cells in function. Two types of conductile fibers can

be identified: narrow diameter fibers and wide diameter fibers, which are also called **Purkinje fibers**.

c. Endocrine cardiocytes. As a result of electron microscopy, it has been known for a long time that certain atrial cardiocytes contain unique cytoplasmic “bodies” (Kisch, B. *Exp Med Surg* 14:99-112, 1956), which were subsequently called *atrial-specific granules* (Jamieson, JD & Palade, GE. *J Cell Biol* 23:151-172, 1964). These granules have been found to contain peptide hormones with natriuretic, diuretic, and vasorelaxant activities.

i. ANP. From experiments using atrial extracts, it was proposed that a substance in the atrial granules caused marked sodium excretion (natriuresis) and increased urine volumes (diuresis) when administered to animals (De Bold, AJ et al. *Life Sci* 28:89-94, 1981). This substance, first called atrial natriuretic factor (ANF), is now known as **atrial natriuretic peptide (ANP)**. It is found primarily in atrial cardiocytes (right>left), but it has also been found in ventricular myocytes, and conductile myocytes (Hansson, M. *Microsc Res Tech* 58:378-386, 2002).

ii. BNP. Another natriuretic peptide, first discovered in brain (Sudoh, T et al. *Nature* 332:78-81, 1988), was called **brain natriuretic peptide (BNP)**. Similar in overall function to ANP, this peptide has also been found in atrial myocytes at a low level, but is predominantly localized in the ventricular myocytes (Hosoda, K et al. *Hypertension* 17:1152-1155, 1991; Ogawa, Y et al. *Circ Res* 69:491-500, 1991).

Extracellular matrix

1. External lamina is the basal lamina-type structure that immediately surrounds the sarcolemma of cardiac muscle cells. It is a secretory product of cardiac myocytes and consists mainly of type IV collagen, laminin, and perlecan (heparan sulfate proteoglycan).

2. Other components. Differential contributions to other matrix components by cardiocytes and cardiac fibroblasts has not been resolved. These issues are important as they relate to fibrosis in the ageing heart.

3. Connective tissue. Individual cardiocytes are surrounded by a delicate connective tissue framework containing an abundant capillary network for delivering oxygen and nutrients to each cell. Although some authors use the term endomysium for this CT, this term is traditionally reserved for skeletal muscle. The cardiac CT is continuous with the cardiac skeleton.

Control of cardiac muscle.

1. Neural influence. Cardiac muscle does not require neural input for activation, but its activity is modulated by the sympathetic (increased contractile activity) and parasympathetic (decreased activity) divisions of the autonomic nervous system (ANS).

2. Cardiac conduction system. Intrinsic (endogenous) control of cardiac muscle function is mediated by the **impulse conducting system**, which consists of conductile cardiac myocytes arranged in the following order: **S-A node, internodal fibers, AV node, AV bundle with right and left branches**, and **subendocardial branches**. Conductile myocytes, as their name implies, are specialized for conduction rather than contraction. In this respect, these unique cardiac muscle cells are more like nerve cells in function.

a. Sinu-atrial (S-A) node (also sinoatrial node, sinus node) is located in the right atrium at the junction with the superior vena cava; it contains specialized muscle cells that spontaneously depolarize rhythmically to initiate each heart beat. This area is known as the **cardiac pacemaker**.

i. Gap junctions provide electrical coupling at intercalated disks to propagate depolarizations.

ii. Internodal tracts of conductile cardiocytes spread pacemaker impulses to atrial myocytes as well as to the A-V node.

b. Atrioventricular (AV) node is located in the right atrium near the AV junction between the tricuspid valve and the orifice of the coronary sinus. The impulse is then forwarded to the ventricles after a short delay of about 0.1 seconds.

c. Ventricular fibers

i. AV Bundle (bundle of His). From the A-V node, a trunk of conduction fibers run through the fibrous skeleton of the interventricular septum where they divide into two branches.

ii. Right and left bundles (bundle branches) are smaller branches of the main AV bundle.

iii. Subendocardial branches, which transmit impulses to ventricular contractile myocytes, are the terminal branches of the conductile apparatus and consist of much larger diameter conductile fibers that ramify to the ventricles from the respective right and left bundle branches. These fibers, which are called **Purkinje**

fibers, are composed of cells that are characterized by their large amount of cytoplasm containing fewer myofibrils and abundant mitochondria and glycogen. N.B.: some authors use the term Purkinje fiber loosely to describe all of the conductile myocytes from the nodal fibers to their terminations. However, the usage here will be traditional and restrict the term Purkinje fibers to the wide diameter conductile myocytes. Actually, the other conductile myocytes, from S-A node to AV bundles are typically smaller in diameter than normal myocytes.

Location of cardiac muscle. Cardiac muscle is found mainly in the myocardium of the heart. However, it also occurs in the walls of some veins associated with the heart, e.g., coronary sinus (Coakley, JB & Summerfield King, T. *J Anat* 93:30-35, 1959).

Regeneration and repair. Until recently, it has been widely accepted that cardiac muscle tissue is in not capable of renewal or repair (e.g., Soonpaa, MH & Field, LJ. *Circ Res* 83:15-26, 1998). This dogma has been questioned by recent studies, which have provided evidence that cardiac myocytes, under certain circumstances, can exhibit limited regeneration (e.g., Arbustini, E et al. *Am J Cardiol* 72:608-614, 1993; Quaini, F et al. *Circ Res* 75:1050-1063, 1994; Kajstura, J et al. *Proc Natl Acad Sci USA* 95:8801-8805, 1998; Beltrami, AP et al. *N Engl J Med* 344:1750-1757, 2001).

a. Stem cells. Whether regeneration of cardiac muscle results from 1) terminally differentiated cardiac myocytes becoming mitotically active, 2) activation of a quiescent population of cardiac stem cells (from within heart muscle, CT or blood vessels), or 3) from blood-borne precursors (e.g., bone marrow stem cells) remains to be determined.

b. Clinical implications. Although the notion of cardiac stem cells has generated considerable excitement, it is still clear that the limited regenerative capacity of the heart cannot prevent such conditions as heart failure. However, the concept of stem cell renewal opens the door for potential treatments in the future.

SMOOTH MUSCLE

Smooth muscle cells are typically elongated cells that are described as fusiform [L. *fuscus* =a spindle + *forma* =form] or spindle-shaped, which are tapered at both ends. In some locations (e.g., blood vessels), smooth muscle cells may exhibit branching. Although they contain myofilaments, they are nonstriated and have also been called **plain**

muscle. Each cell is surrounded by an external (basal) lamina and delicate reticular fibers.

1. Size. Smooth muscle myocytes vary in length from 15 μm to as large as 500 μm in the pregnant uterus. They are generally 3-8 μm in diameter.

2. Nucleus. The single centrally-located nucleus is described as “cigar-shaped” in the uncontracted myocyte. In cross-sections, this description means that the nucleus will have a circular profile. Recall that fibroblast nuclei are “surfboard-shaped” so they are flattened in xs. In contracted myocytes, nuclei take on a “corkscrew” shape, which can readily be used as a diagnostic feature.

3. Cytoplasm. In H&E preparations, the cytoplasm is usually uniformly eosinophilic due to the abundance of actin and myosin proteins. In trichrome preparations they stain red in contrast to the blue or green collagen fibers of CT.

4. Locations. Smooth muscle is found in many locations throughout the systems of the body including the walls of hollow organs (e.g., alimentary canal, blood vessels, urinary bladder, uterus, ductus deferens), and in the skin where bands of smooth muscle cells can be found in the **arrector pili** muscles of hair follicles.

5. Syncytial behavior. Although smooth muscle cells are discrete units, like cardiac myocytes they are electrically coupled with each other. Gap junctions, which can be seen with the EM, are the basis of this physiological approximation to a syncytium.

6. Myofilaments. Smooth muscle cells contain actin and myosin filaments, but they are not arranged in the orderly arrays like those found in striated muscle.

a. Arrangement. Actin and myosin filaments course obliquely in the cytoplasm forming a lattice-like arrangement.

b. Z line equivalents. Thin filaments are attached to punctate structures called **dense bodies**, which are located at multiple locations within the cytoplasm and attached to the plasma membrane. Thus, dense bodies serve the function of Z lines of striated muscle.

Extracellular matrix

1. External lamina is the basal lamina-type structure that immediately surrounds the sarcolemma of smooth muscle cells (except at gap junctions). It is secreted by smooth muscle cells and consists of type IV collagen, laminin, and perlecan (heparan sulfate proteoglycan).

2. Argyrophilic reticulum. Adjacent to external laminae, a delicate network of reticular fibers are found between adjacent smooth muscle cells. This network, which is argyrophilic with silver stains, is composed principally of type III collagen fibers. Some authors, particularly in the clinical literature, sometimes use the term endomysium for this layer.

3. Other matrix components. Smooth muscle myocytes also secrete other proteoglycans and elastin. In certain locations (e.g., blood vessels, uterus), they can secrete large amounts of type I collagen and elastin.

Other smooth muscle-like cells. Due to their non-striated appearance, and presence of contractile properties and proteins, several other cell types share some structural and functional characteristics with smooth muscle cells. They include the **myoepithelial cells** that are found in certain epithelial glands, **myofibroblasts** of CT, the specialized **epithelioid cells of the perineurium**, and the **peritubular myoid cells** surrounding the seminiferous tubules in the testes.

Contraction of smooth muscle is slow and sustained. Individual cells may contract completely or a wave of contraction may propagate from one end to the tissue to the other.

1. Mechanism of contraction. A sliding filament mechanism of contraction occurs.

2. Initiation of contraction. Smooth muscle cell contraction may be triggered by various stimuli, including nervous and hormonal.

a. Nervous

i. Extrinsic. In some organs (e.g., ductus deferens), an extrinsic nerve supply to smooth muscle is abundant.

ii. Intrinsic. In smooth muscle of the alimentary tract, rhythmic depolarizations and contraction are controlled by nerve fibers from intrinsic enteric ganglia, which in turn may be modulated by neural input from the ANS.

b. Hormonal. Hormones may also induce or modulate smooth muscle contraction. For example, oxytocin stimulates smooth muscle contraction in the uterus, as well as contraction of the smooth muscle-like myoepithelial cells in mammary glands.

3. Role of calcium. Smooth muscle cells are able to concentrate calcium in their cytoplasm. This ion plays an important role in contraction.

a. Influx of calcium from outside the cell occurs during depolarization of the cell membrane.

b. Sequestering of calcium occurs in either the cell membrane or in the sparse sarcoplasmic reticulum.

Regeneration and repair of smooth muscle appears to be an ongoing process. Myocytes divide by mitosis in many locations including alimentary canal, blood vessels, and the uterus. Undifferentiated mesenchymal cells and pericytes of blood vessels serve as two of the potential sources of stem cells for smooth muscle renewal.

HistoDDx: Histologic Distinctions of Muscle Types

Striated muscle: longitudinal sections. In ls, both skeletal and cardiac muscle fibers exhibit vertical striations perpendicular to the long axes of cells. However, several important differences can be observed. 1) Cardiac muscle branches, skeletal muscle does not. 2) Since skeletal muscle cells are syncytial, and each myofiber has many nuclei, which are arranged at the cellular periphery adjacent to the sarcolemma. Cardiac muscle consists of separate cellular units, which may have one or two nuclei. 3) Finally, cardiac muscle is organized with cells in an end-to-end arrangement. Intercalated disks, which are intercellular junctions that attach cardiocytes together at their ends, are characteristic only of cardiac muscle. It is important to remember that skeletal muscle cells extend the full length of a muscle and therefore do not have intercellular junctions at their extremities. Specializations for attachment of myocytes to the ECM and CT do occur at origins and insertions and along lateral surfaces. However, these specializations are not resolved with the LM.

Striated muscle: cross sections. In xs, skeletal and cardiac muscle present a stippled appearance, which reflects their myofibrillar organization (discrete in skeletal; partial in cardiac). However, since skeletal muscle cells do not branch, they show uniformly oval or polygonal profiles in xs. In contrast, since cardiac muscle is branched, xs profiles exhibit a variety of odd shapes including “figure

8s”. In addition, skeletal muscle nuclei are numerous and located peripherally, whereas the cardiac muscle nucleus is centrally-placed. Often cardiocyte nuclei are lacking from xs profiles since only one (or two) nuclei are present in each cell. Rarely are nuclei lacking from skeletal muscle xs profiles. Although smooth muscle cells contain centrally-placed nuclei, typical myofibrils are absent. Finally, the organization of CT sheaths can be helpful in a diagnosis. While typical skeletal muscles display distinctly subdivided CT compartments (perimysium, etc), other varieties seldom show this orderly arrangement.

Smooth muscle is often more likely to be confused with collagenous CT than with striated muscle, since both smooth muscle and dense CT stain well with eosin and contain elongated nuclei. However, smooth muscle tissue is more cellular than collagenous CT and therefore has more nuclei. Smooth muscle is also more organized with less intercellular spaces and with less intercellular matrix (e.g., collagen) than connective tissue. Finally, the shape of the nucleus is important in a ddx. In longitudinal section, both have elongated nuclei, but fibroblast nuclei are often more heterochromatic, especially in resting cells. If the smooth muscle cells are contracted, their nuclei will exhibit the characteristic “corkscrew” shape. In xs, the fibroblast nucleus is flattened in one direction since it has a “surfboard” shape overall; the smooth muscle nucleus will generally be circular since it has a “cigar” shape overall.

Table 8-1. Comparisons of Important Characteristics of Muscle Types

	Skeletal	Cardiac	Smooth
Syncytium			
Morphologic (Cellular)	Yes	No	No
Physiologic (Tissue)	No ¹	Yes	Yes
Branching of fibers	No	Yes	Some
Size (myocyte)			
Length	few mm-meter	10-100µm	15-500µm
Diameter	10-100µm	10-15µm	3-8µm (mid-part)
Striations	Yes	Yes	No
Sarcomeres	Yes	Yes	No
Myofibrils	Yes	Partial	Quasi
Nuclei			
Number	Many	1-2	1
Position in cell	Peripheral	Central	Central
T-tubules			
Location	A-I junction	Z line	none (caveolae)
With SR	=triad	=diad	
Intercellular junctions²	No	Yes	Yes
External lamina	Yes	Yes ³	Yes ²
Blood vessels	Many	Many	Fewer
Motor control (typical)	Voluntary	Involuntary	Involuntary
Body locations	Head, neck, body wall, extremities; some organs	Heart, adventitia of vena cava and pulmonary vein	Walls of viscera, blood & lymph vessels; skin; eye

¹Although individual skeletal myocytes are syncytial (i.e., they form by the fusion of many myoblast precursors to form large multinucleate mature cells), they themselves are not coupled by gap junctions to adjacent myocytes as is the case for cardiac and smooth muscle cells. The latter cells, which share extensive information via gap junctions, represent an arrangement that can be termed a “physiologic syncytium”.

²Skeletal muscle cells have junctions with nerve cells (neuromuscular junctions), but not with other skeletal muscle cells; in contrast, junctions between contractile cells occur in both cardiac and smooth muscle.

³External lamina is discontinuous at sites of communicating junctions.
