Structure and Function of Blood Vessels

Scientific Foundations

Learning Objectives

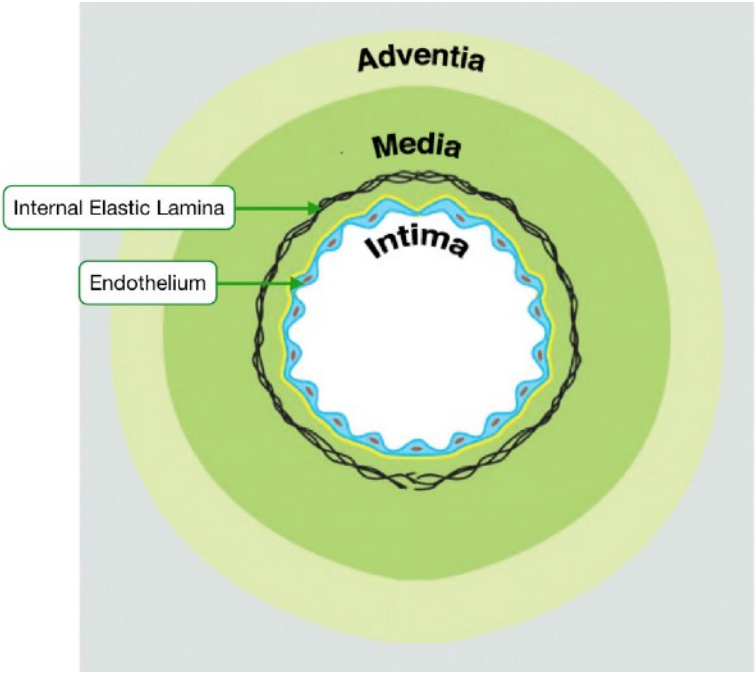
1. Students should be able to list the differences in structure and permeability between the three types of endothelia.
2. Students should be able to list the components of endothelium that determine its permeability.
3. Students should be able to describe two molecular mechanisms that increase the permeability of endothelium.
4. Given a histological image, students should be able to identify the different types of blood vessels and explain the role each plays in the conduction of blood and distribution of nutrients.
5. Given an histological image of a large artery, students should be able to describe the function of each layer of the wall of the artery and the components and cells in each layer.
6. Given an electron micrograph of a capillary, students should be able to determine whether the capillary is continuous, fenestrated or discontinuous.

Walls of a Blood Vessel

Depending on their size, blood vessels can contain three distinct layers

- The **tunica intima** is thin and composed of endothelial cells and their underlying supporting tissue, which includes the basement membrane and internal elastic lamina. The internal elastic lamina is composed of elastic fibers.
- The **tunica media** is the largest portion of the wall and is composed of elastic fibers, smooth muscle and collagenous tissue.
- The **tunica adventitia** is the outermost component of the arterial wall. It contains mostly connective tissue in the form of collagen and a few small blood vessels called vasa vasorum that supplies the cells that make up the arterial wall.

These three layers will be visible in large arteries and veins, but as the diameter of the vessel decrease, the adventitia and media layers become thinner. In the smallest blood vessels, capillaries, only endothelial cells, a basement membrane and an occasional support cell are found.



Blood vessels comprise three functional layers.

Endothelial cells perform several critical functions

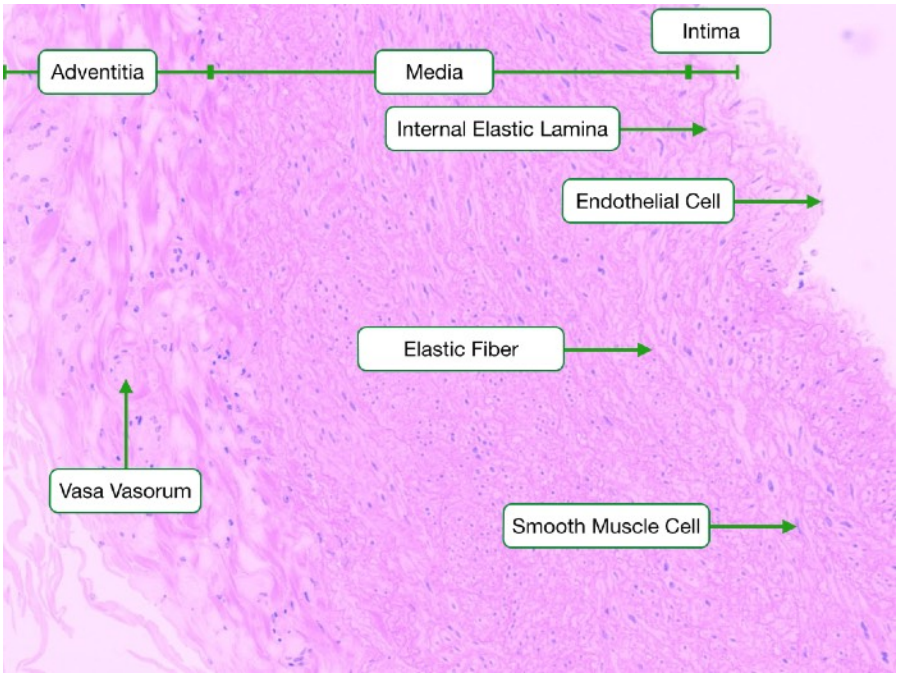
Endothelial cells are the only cell to directly contact blood and as consequence they perform a variety of physiological functions.

- Regulate permeability of vessels: control passage of proteins and small molecules.
- Regulate immune response: entry site into tissue for white blood cells.
- Initiate growth of new blood vessels
- Detect and respond to changes in blood pressure and flow: signal to underlying smooth muscle.
- Regulate clotting.
    * Inhibit platelet aggregation.
    * Secrete Von Willebrand part of clotting cascade.
- Modify plasma proteins.
    * Angiotensin converting enzyme: increase blood pressure

Elastic Artery: Aorta Wall

This image shows the wall of an elastic artery. Note the three layers: adventitia, media and intima. Elastic arteries are notable for the numerous elastic fibers in their tunica media. In H&E-stained samples, the elastic fibers will appear as pink, wavy lines. The elastic fibers allow elastic arteries, such as the aorta, to stretch during systole to accommodate a large volume of blood, and then contract during diastole to push the blood downstream through cardiovascular system. Note the internal elastic lamina that separates the media from intima layer. The internal elastic lamina is rich in elastic fibers and provides flexibility to the intimal layer.

The tunica adventitia contains mostly connective tissue in the form of collagen and a few small blood vessels called vasa vasorum that supplies the cells that make up the arterial wall.



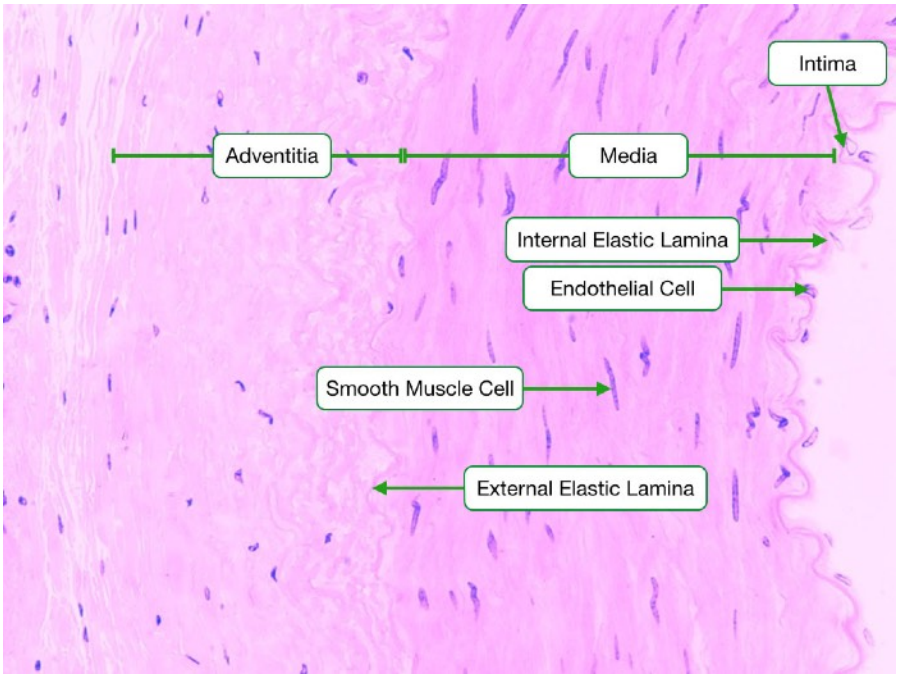
The aorta is an elastic artery that contains numerous elastic fibers in the media.

Muscular Artery Wall

Muscular arteries continue from elastic arteries and control the distribution of blood throughout the body. The tunica media of muscular arteries contains fewer elastic fibers and more smooth muscle cells than elastic arteries. Note the prominent internal elastic lamina and external elastic lamina which separates the tunica media from tunica adventitia and is also composed of elastic fibers.

Muscular arteries control the distribution of blood to different parts of the body. The smooth muscle cells in the medial layer contract to decrease the lumen of the artery and decrease blood flow or relax to increase the diameter of the lumen and the amount of blood flow. Smooth muscle

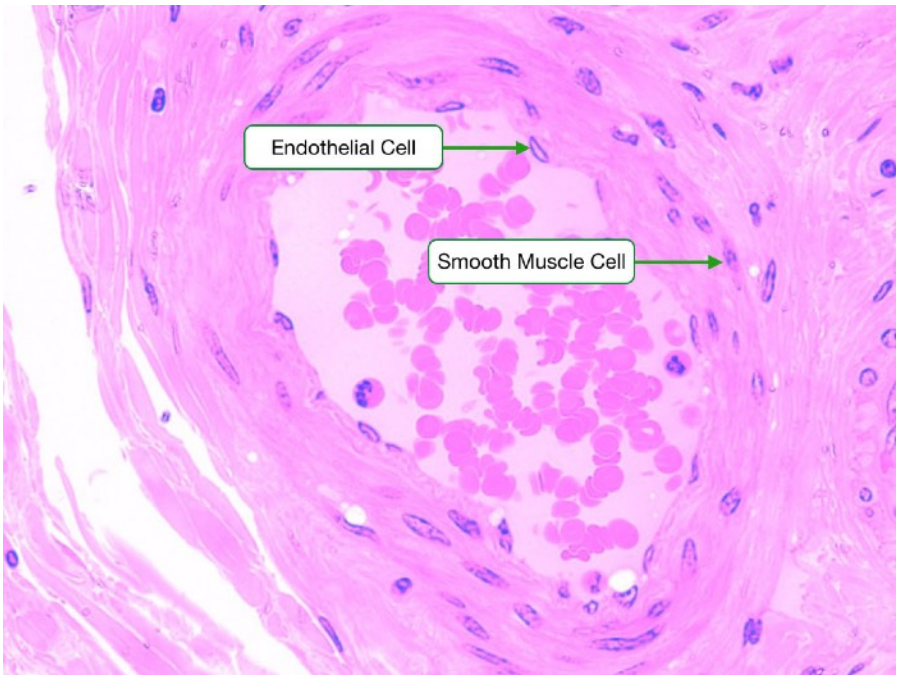
cells are also responsible for synthesizing all the protein components in the walls of blood vessels, including collagen, elastic fibers and proteoglycans.



Muscular arteries contain more smooth muscle and fewer elastic fibers in the media layer.

Small Muscular Artery

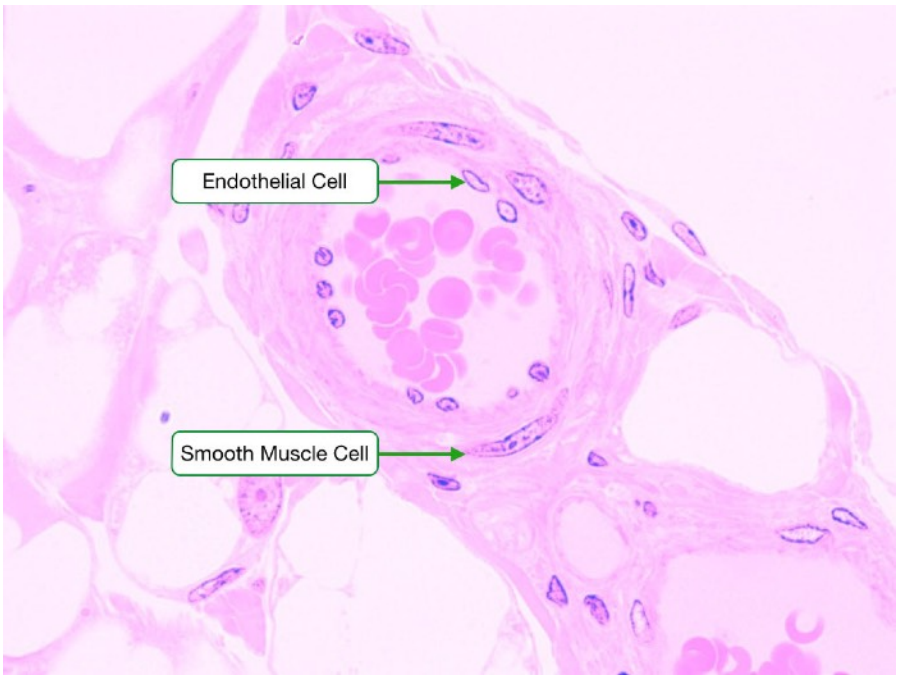
As muscular arteries branch and decrease in size, the number of layers of smooth muscle cells in the tunica media decreases. Also, the internal and external elastic laminae become much less prominent.



Small muscular arteries contain fewer layers of smooth muscle.

Arterioles

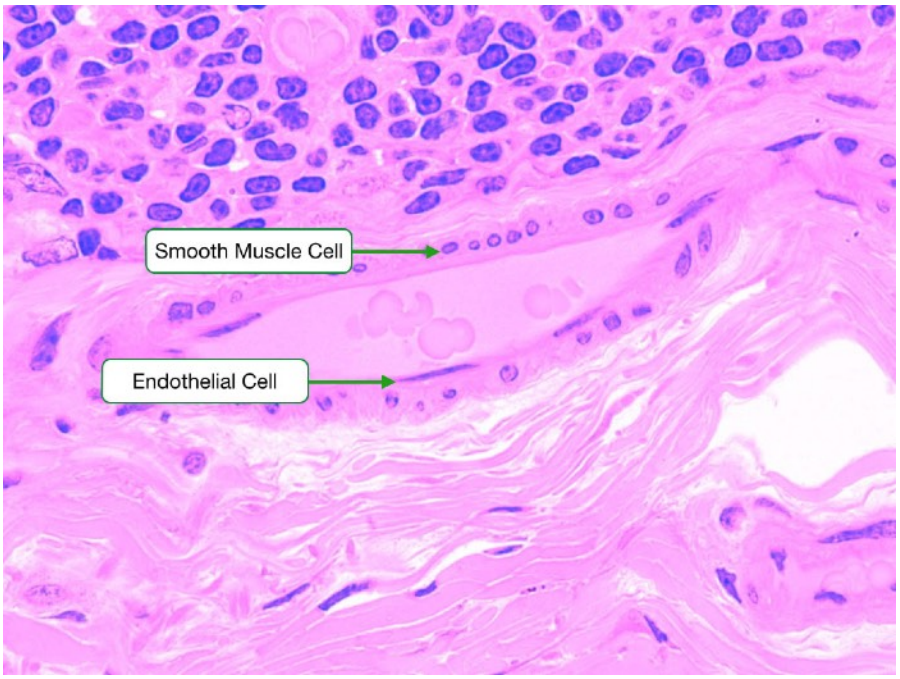
In arterioles, the tunica media contains only one or two layers of smooth muscle cells. Contraction of the smooth muscle cells constricts the lumen of the arteriole, increasing vascular resistance and reducing the flow of blood into capillary beds. The arterioles generate the largest resistance to blood flow in the circulatory system.



Arterioles control blood flow into capillaries and create the highest resistance in the circulatory system.

Arteriole, Longitudinal Section

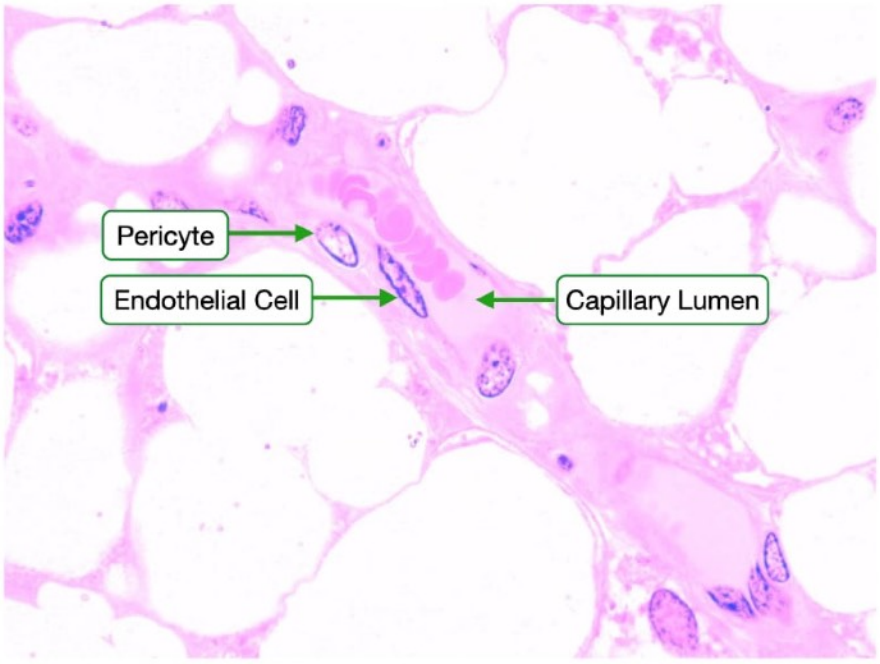
This image shows an arteriole in longitudinal section. The arteriole has a single layer of smooth muscle cells. Note the orientation of the smooth muscle cells in longitudinal section versus cross section.



Arterioles have one or two layers of smooth muscle cells.

Capillary

Capillaries contain a single layer of endothelial cells and their basement membrane. The lumen of capillaries is so narrow that red blood cells have to pass in single file. The thin walls of capillaries facilitate exchange of gases and small molecules between the bloodstream and surrounding tissue. Capillaries are often associated with pericytes which are contractile cells that regulate the activity of endothelial cells through cell adhesion proteins and gap junctions.

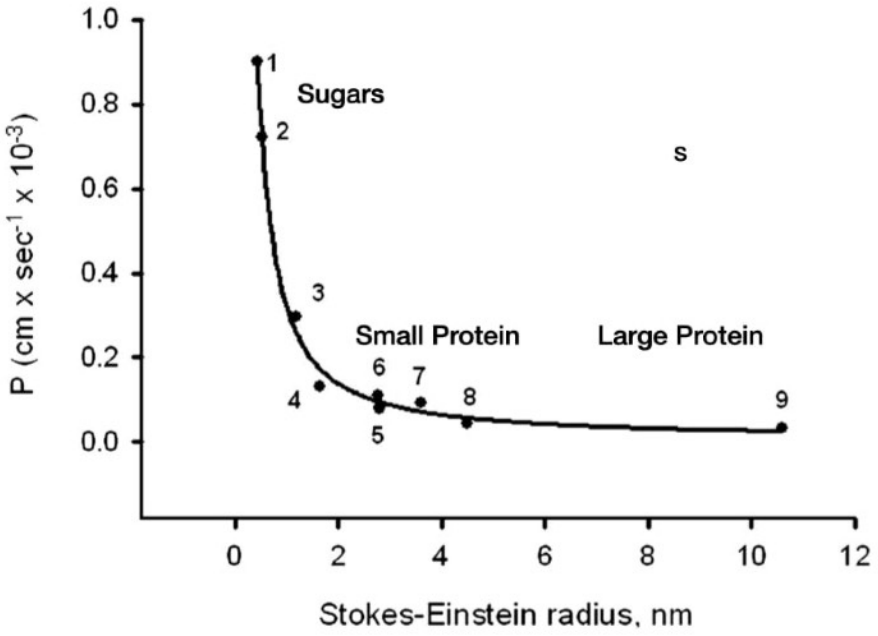


Capillaries contain a single layer of endothelial cells and the occasional pericyte.

Permeability of Capillaries

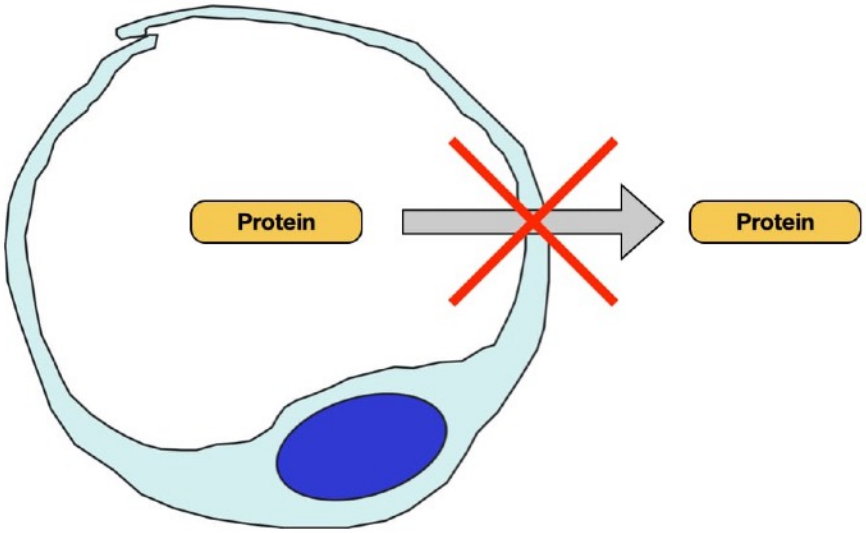
One of the most important roles of a capillary is to control what passes from the blood into the surrounding tissue and fluid.

The permeability of capillaries displays two phases. The first is a size-selective phase in which the permeability of molecules is directly related to their size. This holds for small molecules like sugars. Larger molecules, such as proteins, show permeability that is not size restrictive. That is, all molecules above a certain size (about 2 nm) are equally permeable. This suggests there are two different ways for solutes to pass across a capillary.



Small molecules and ions diffuse across capillaries but proteins are restricted in most capillaries.

Most capillaries restrict the passage of proteins to prevent excessive fluid loss into tissues. Blood is under hydrostatic pressure that pushes fluid out of capillaries. Blood vessels are leaky to most ions, so ions can not be used to generate an osmotic pressure difference between blood and surrounding tissue. To counter the hydrostatic pressure, blood retains a high concentration of protein to generate oncotic pressure that draws water into the vessel. Loss of protein out of vessels would decrease oncotic pressure, allowing fluid to leak into surrounding tissue and causing edema. Some proteins, however, must exit the blood to reach the surrounding tissue. These could be antibodies or proteins that escort hormones or lipids. So capillaries cells a mechanism to get specific proteins out of the blood.



Capillaries restrict the diffusion of protein to maintain oncotic pressure.

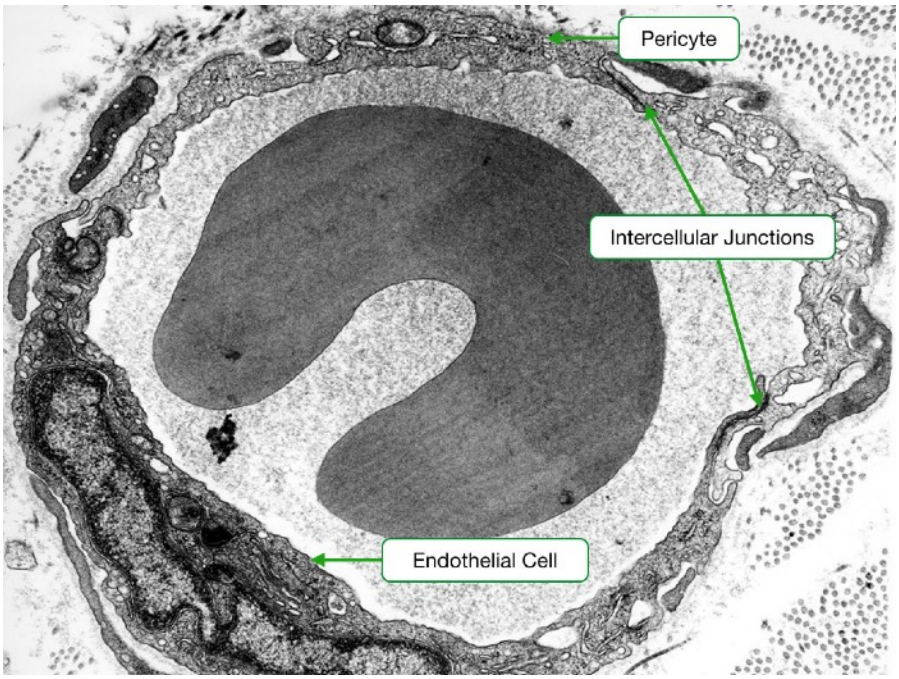
There are four primary components that restrict the diffusion of material between the blood and interstitial fluid: the structure of the endothelial cells, junctional complexes, basement membrane and glycocalyx.

Structure of Endothelial Cells

Because endothelial cells line the entire wall of capillaries, their structure plays a critical role in controlling the movement of molecules and proteins across capillaries. There are three structural types of capillaries: continuous, fenestrated and discontinuous.

Continuous Capillary, Electron Micrograph

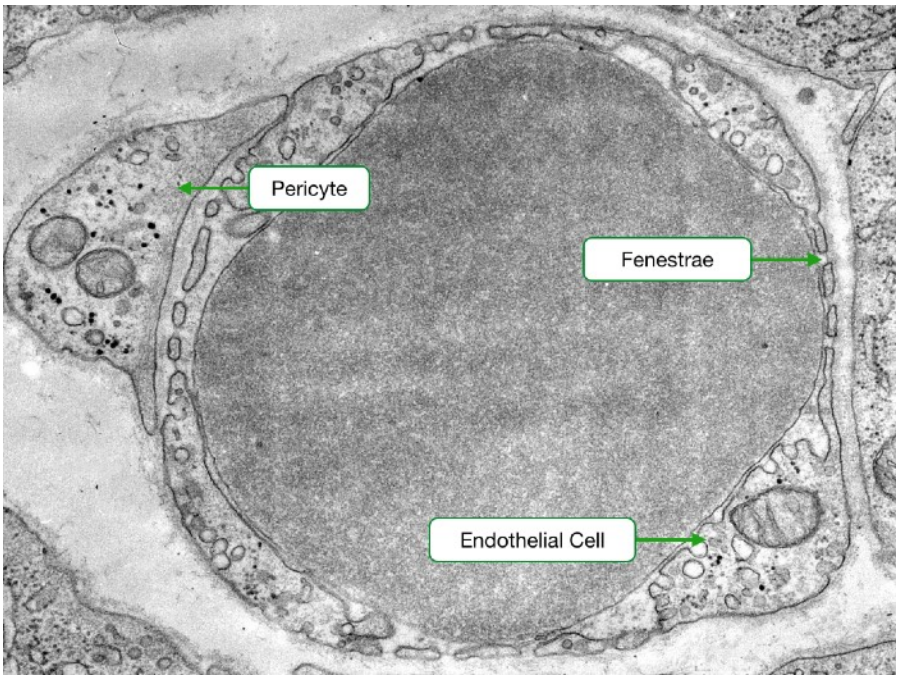
Endothelial cells in continuous capillaries completely enclose the lumen of the blood vessel. The only gaps are the junctions between adjacent endothelial cells where small molecules can diffuse between the bloodstream and surrounding tissue. In addition, a basement membrane completely surrounds the endothelial cells. Consequently, continuous capillaries are the most restrictive. Continuous capillaries are prominent in adipose and muscle tissue and in the brain.



Continuous capillaries contain endothelial cells that only allow passage through intercellular junctions.

Fenestrated Capillary, Electron Micrograph

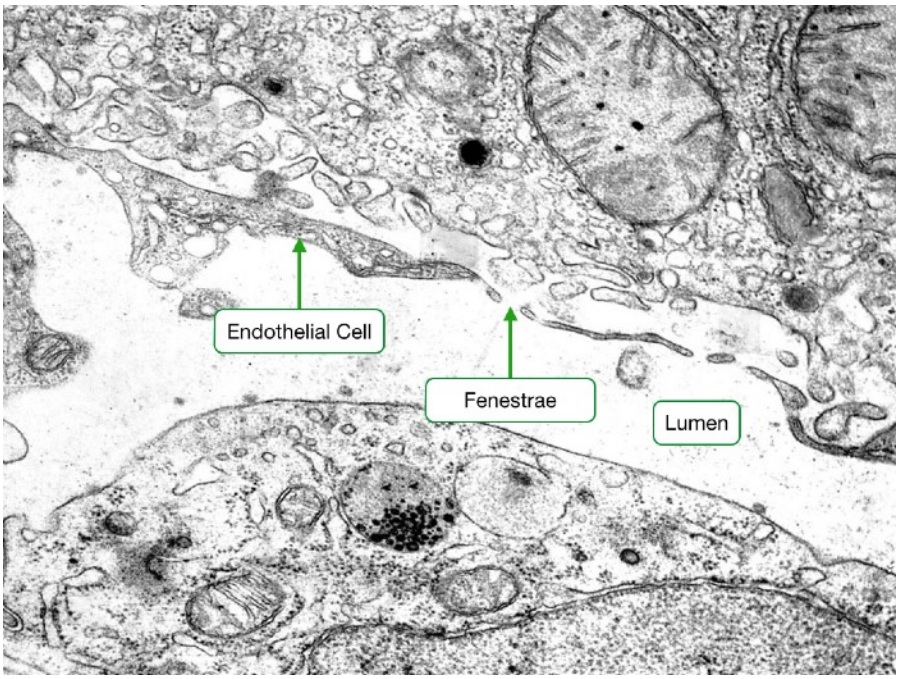
Fenenstrated capillaries are more permeable than continuous capillaries because the endothelial cells contain holes or fenestrae that allow small molecules and certain proteins to pass through the endothelium. Barely visible in the fenestrae are electron-dense lines known as diaphragms that functions as a filtration barrier. In addition, a basement membrane completely surrounds the endothelial cells Fenestrated capillaries are prominent in the kidney, intestine and endocrine glands.



Fenestrated capillaries have endothelial cells with several gaps for diffusion of solutes.

Discontinuous Capillary, Electron Micrograph

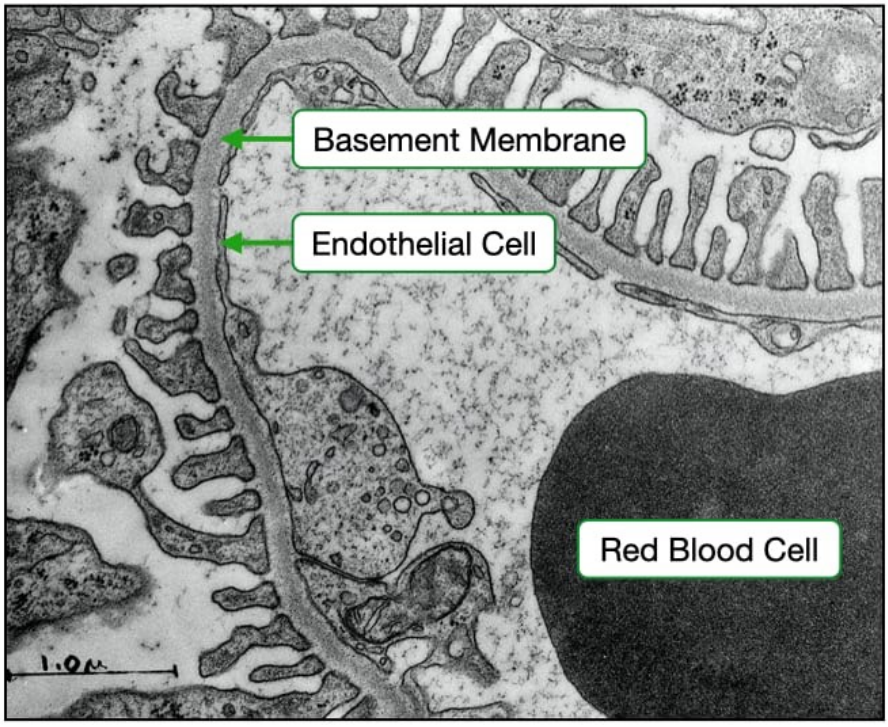
Discontinuous capillaries are the leakiest of the three types of capillaries. Similar to fenestrated capillaries, discontinuous capillaries contain gaps, but the gaps here are larger than in fenestrated endothelia and lack diaphragms. These gaps allow proteins to diffuse freely across the endothelium. Note also that there is no basement membrane beneath discontinuous endothelia, whereas both continuous and fenestrated capillaries contain a basement membrane. The lack of a basement membrane removes another barrier to protein diffusion (see below). Discontinuous endothelia are prominent in the liver, spleen and bone marrow and are often called sinusoids in these organs.



Discontinuous capillaries contain large pores and lack a basement membrane.

Basement Membrane

Similar to epithelia cells, endothelial cells in most capillaries rest on a basement membrane. The basement membrane of endothelium provides structural support and endothelial cells attach to the basement membrane via integrins. In addition, the basement membrane also inhibits diffusion of protein because it is strongly negatively charged. The basement membrane contains a lot of proteoglycans and the sugar groups on the proteins are all negatively charged. Albumin which is the most abundant protein in blood is negatively charged and therefore does not readily diffuse across the basement membrane. The thickness of the basement membrane varies between organs. Capillaries in the kidney have a thick basement membrane to prevent loss of protein into urine.

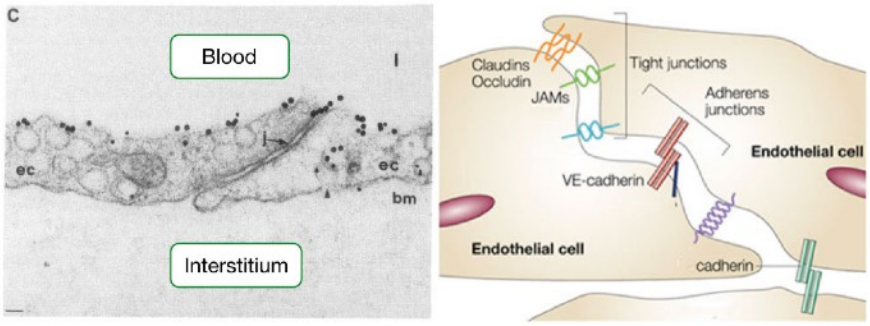


Basement membrane is a negatively-charged barrier on the basal surface of endothelial cells.

Junctional Complexes

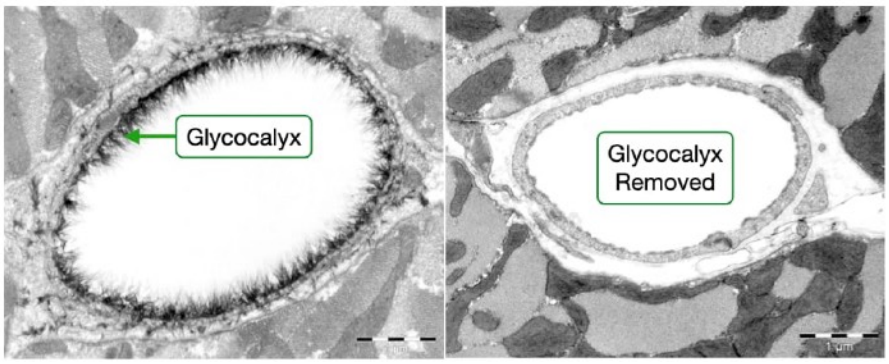
The junctional complexes are similar to those of other epithelial cells. They contain tight junctions and adhering junctions. The tight junctions contain claudins that restrict the passage of small molecules and ions. The type of claudin expressed determines the restrictiveness of the junction. Most endothelial cells allow passage of ions and small molecules but endothelial cells in the brain express claudin 5 that make that endothelium extremely restrictive to passage of small molecules. Endothelial cells are also held together by adhering junctions. These interactions are mediated by a special type of cadherin called VE-cadherin by function similar to cadherins in other epithelia. Cadherins are critical for preventing fluid loss from vessels. Injecting antibodies against extracellular domain of cadherins disrupts connections between endothelial cells leading to edema.

In the image on the left, albumin has been injected into the lumen and appears as black dots. Note how the albumin is confined to the lumen and does not pass through the junction between endothelial cells.



Junctional complexes restrict paracellular diffusion between endothelial cells.

A more recently discovered component of the permeability barrier is glycocalyx. Glycocalyx forms a brush like structure on the apical surface of endothelial cells. Glycocalyx can extend several hundred nanometers into the lumen of the vessels. Glycocalyx was first thought to primarily prevent red blood cells from sticking to the surface of endothelium. Recent work has shown that glycocalyx is a critical component that prevents diffusion of protein across endothelium. Enzymatic removal of glycocalyx leads to edema in several tissues. Glycocalyx is composed of a proteoglycans and GAGs. which gives glycocalyx a negative charge. The measure space between proteoglycans is 20 nm suggesting that glycocalyx functions in part like a sieve to restrict passage of larger proteins and macromolecules.



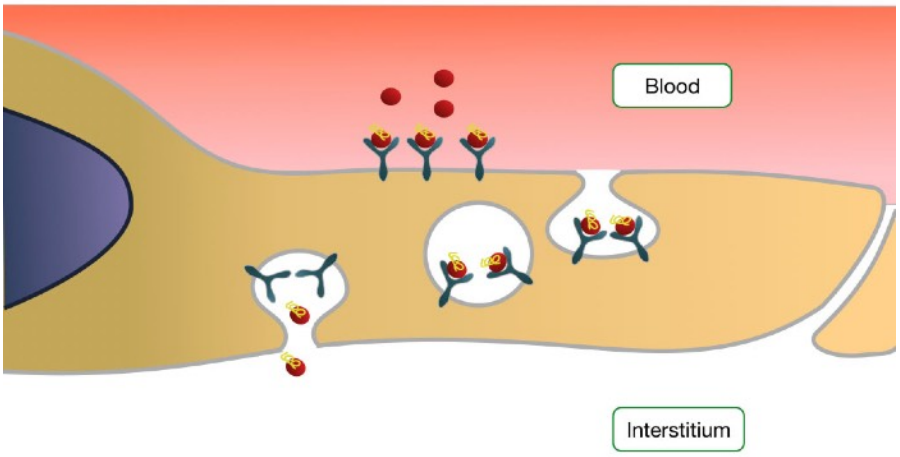
Glycocalyx restricts diffusion of large, negatively charged molecules.

Transcytosis

Certain proteins must be able to cross from the blood into the surrounding tissue. For example, albumin, which the body wants to keep in the blood to maintain oncotic pressure, is a carrier for certain hydrophobic molecules, such as cholesterol. To get cholesterol from the blood into tissues, the endothelium must allow passage of albumin that is bound to cholesterol while preventing free albumin from crossing. The endothelium accomplishes this through transcytosis.

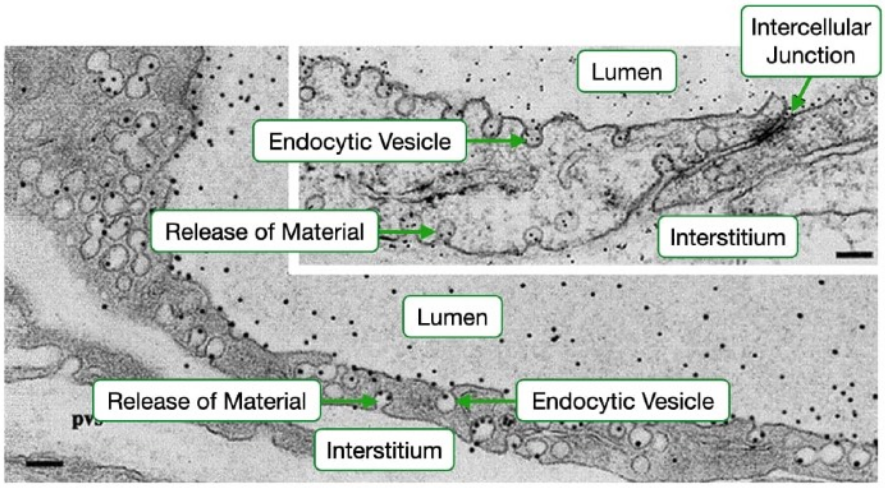
Transcytosis is a process through which cells internalize material through endocytosis on one side and release that material on the opposite. Endothelia cells use transcytosis to move specific proteins from the blood into the surrounding tissue. For example, endothelial cells express on

their surfaces that face blood (abluminal) a receptor that binds albumin only when it is associated with cholesterol. Endothelial cells endocytose the receptor-albumin complex into vesicles. The pH of the vesicles decreases to dissociate albumin from its receptor. The vesicle then fuses with the basal surface to release albumin into the surrounding tissue.



Receptor-mediated endocytosis transports specific proteins across endothelium via transcytosis.

This image reveals the process of transcytosis. The black dots represent labeled albumin that was injected into the blood vessel. The albumin is being endocytosed from the lumen and transported across the cytoplasm of the endothelial cell and then released on the basal side of the endothelial cell into the interstitium. The inset image shows the junction between two endothelial cells and illustrates how the tight junction prevents diffusion of albumin.

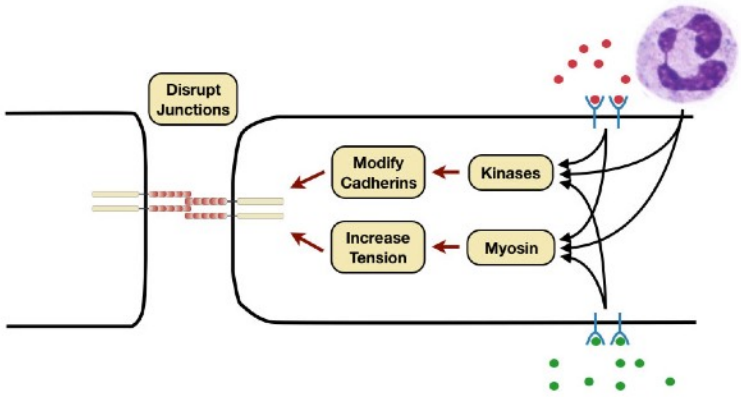


Transcytosis mediates movement of proteins across endothelial cells.

Increasing Permeability During an Immune Response

Occasionally, the junctional complexes need to be loosened to allow fluid, protein and other molecules to diffuse more freely from blood into surrounding tissue. For example, during an infection of a tissue or organ, an immune response requires fluid, protein and even cells to flow from blood into the site of infection. To increase permeability, endothelial cells loosen their junctional complexes by at least two mechanisms.

The first mechanism to weaken interactions between endothelial cells involves increasing intracellular tension that pulls apart endothelial cells. Recall that myosin filaments pulling on actin filaments causes the cells to shrink. Endothelial cells activate myosin which pulls on filaments anchored to the cell membrane. The tension created pulls the cell membrane away from the adjacent cell, increasing the size of the gap between endothelial cells.



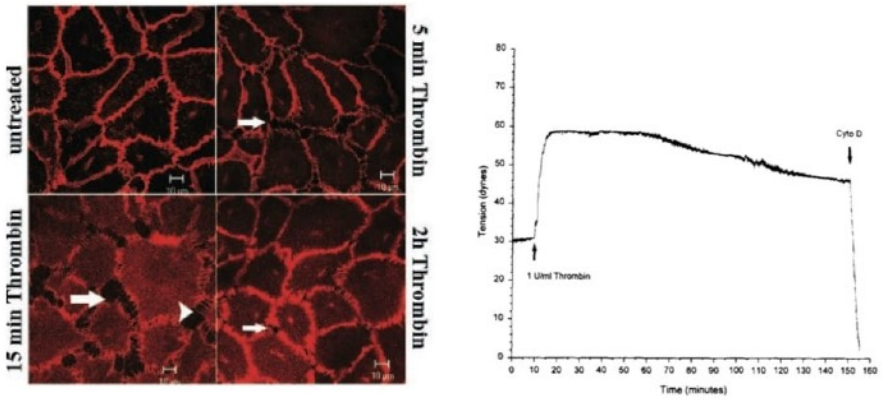
Inflammatory molecules and immune cells activate signaling pathways that disrupt junctions.

The second mechanism involves weakening the interactions between cadherins in adhering junctions. Endothelial cells activate pathways that modify (via phosphorylation) cadherins that

cause them to dissociate from actin filaments. Recall that association with actin filaments clusters cadherins to create strong intercellular connections. Endothelial cells also modify cadhreins so that they become targets for endocytosis. Consequently, the number of cadherins on the cell surface is reduced.

During an immune response, immune cells in the tissue and circulatory system release signaling molecules that activate both pathways in endothelial cells, increasing the permeability of the endothelium to allow fluid, protein and eventually cells to cross the endothelium.

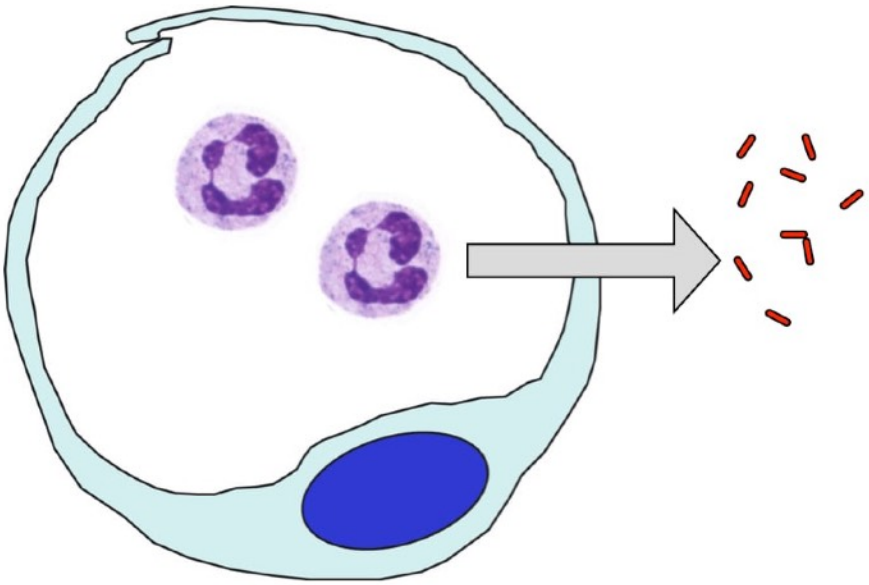
A number of agents increase the permeability of endothelium, including thrombin, bradykinin and histamine. The images on the left show the results of treating endothelial cells with thrombin. The red is stain for junctional protein on the cell membrane. Note how the stain is continuous around the endothelial cells and there are no gaps between cells. 5 minutes after thrombin, small gaps are observed in the junctional complexes. At 15 minutes, spaces are seen between cells. The graph on the right shows that addition of thrombin increased the tension within cells. The tension could be relieved by adding agents that depolymerize actin filaments, suggesting that tension was generated by an actin dependent process.



Thrombin increase vascular permeability through tension on adhesion junctions.

Transmigration of White Blood Cells

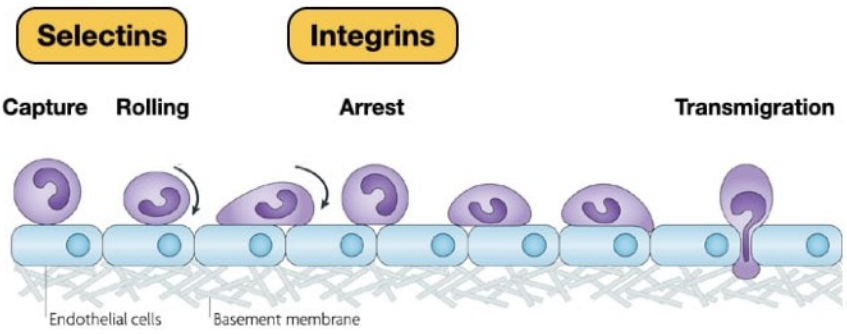
Not only must specific proteins exit the blood but immune cells must also cross the endothelium to enter the surrounding tissue to fight infections. Given the significantly larger size of cells compared to proteins, this would seem to be a near impossible challenge. Endothelial cells play two important roles in this process. First, they attract immune cells to the sites of infection. Second, they allow immune cells to pass across and enter the surrounding tissue.



Immune cells must pass across endothelial cells to reach sites of infection.

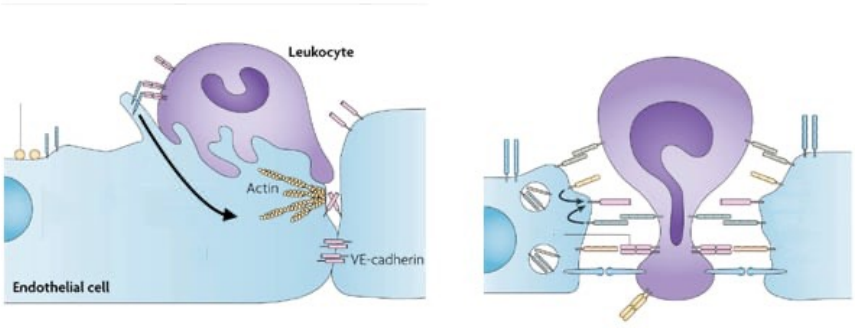
How do immune cells cross endothelium that is usually so restrictive to small molecules? Immune cells cross endothelium in three observable steps. First is rolling where immune cells roll along the surface of endothelial cells whereas red blood cells go flying by. The second is adhesion where immune cells stick to the surface of endothelial cells and don't move. Finally, immune cells are seen to squeeze across endothelium to enter the surrounding tissue.

The first step, rolling, is mediated by a set of proteins called selectins. Endothelial cells express selectins on their plasma membrane in response to infection. The selectins on endothelial cells interact with selectins on immune cells to capture the immune cell to the surface of the endothelium. The interaction between selectins is weak so that immune cells don't stay in one place but are pushed along the surface of the endothelium by the flow of blood. Next, chemokines and chemoattractants released by cells in the tissue in response to infection trigger the expression of integrins on the surface of immune cells. These integrins bind proteins on the surface of endothelial cells. The interaction mediated by integrins is much stronger and locks the immune cell to the surface of endothelium. Finally, the immune cell squeezes between endothelial cells to enter the surrounding tissue.



Selectins and integrins mediate rolling and adhesion of immune cells.

How do immune cells squeeze between endothelial cells? They appear to activate myosin filaments within endothelial cells that generates contraction and weakening of the junctional complexes. This separation between neighboring endothelial cells allows immune cells to insert extensions of their cell membrane between the endothelial cells. The immune cell expresses on its surface proteins that bind to the receptors that make up the junctional complexes of endothelial cells. These interactions likely guide the immune cell across the endothelial cell. After passing across the endothelial cells, the immune cells must still penetrate the basement membrane. Immune cells express a variety of proteases that degrade the structural components of the basement membrane and allow it to pass into the tissue.



Leukocytes disrupt adhesion junctions to migrate across endothelium.

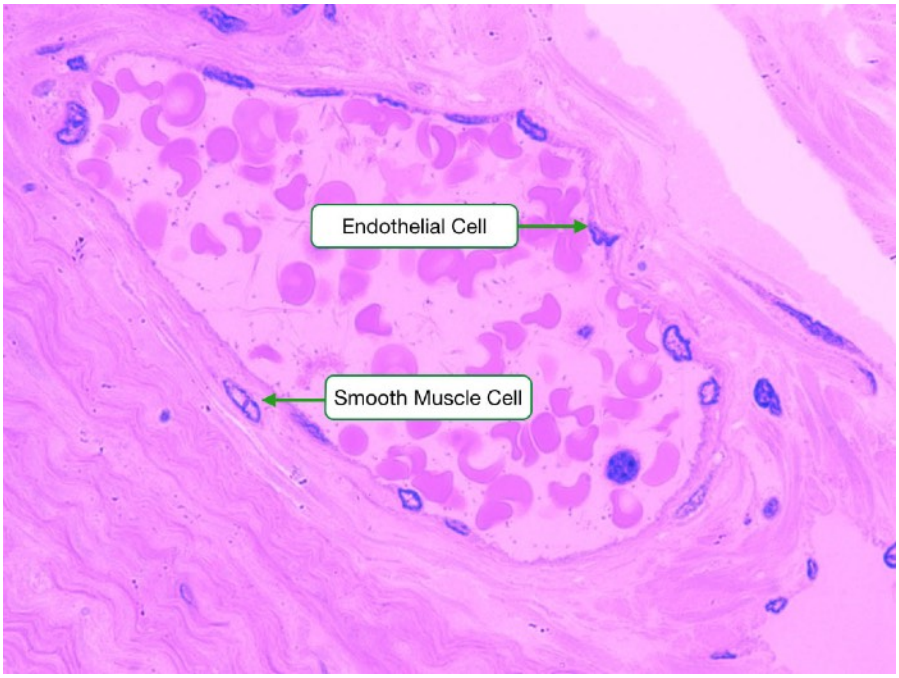
Venous System

The venous system collects blood from capillary beds and returns it to the heart. The venous system is a low pressure system and can store large volumes of blood.

Venule

This image of a venule shows several of its characteristic features. Identify its endothelium and narrow layer of smooth muscle cells. Small venules are usually surrounded by pericytes, and

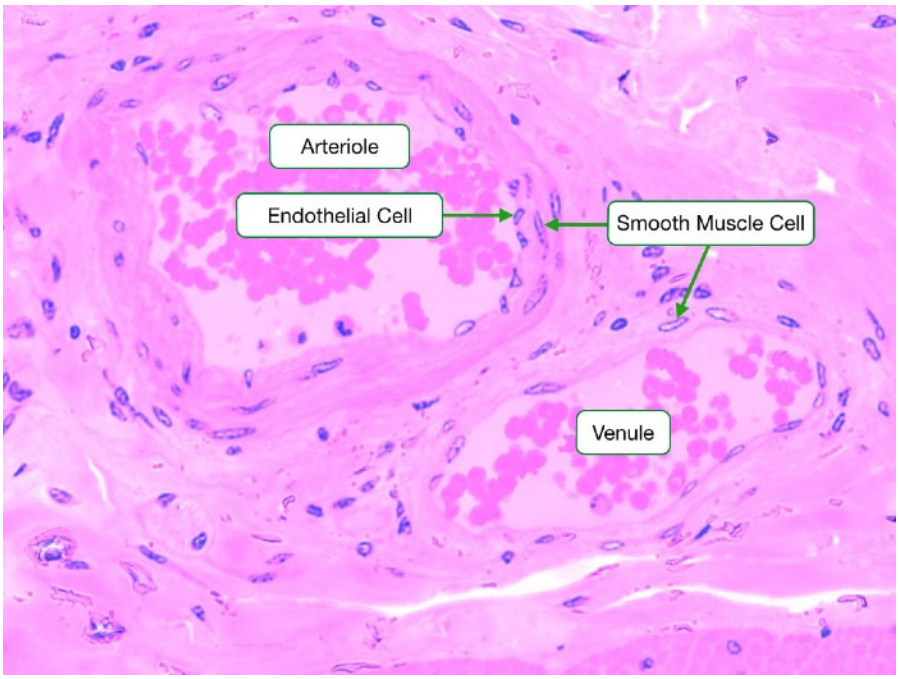
larger venules are surrounded by smooth muscle. Venules collect blood from the capillary beds and play a critical role in immune responses to infection as they are the sites where immune cells cross from the blood into the surrounding tissue.



Venules have an endothelium and relatively thin layer of smooth muscle.

Artery and Venule

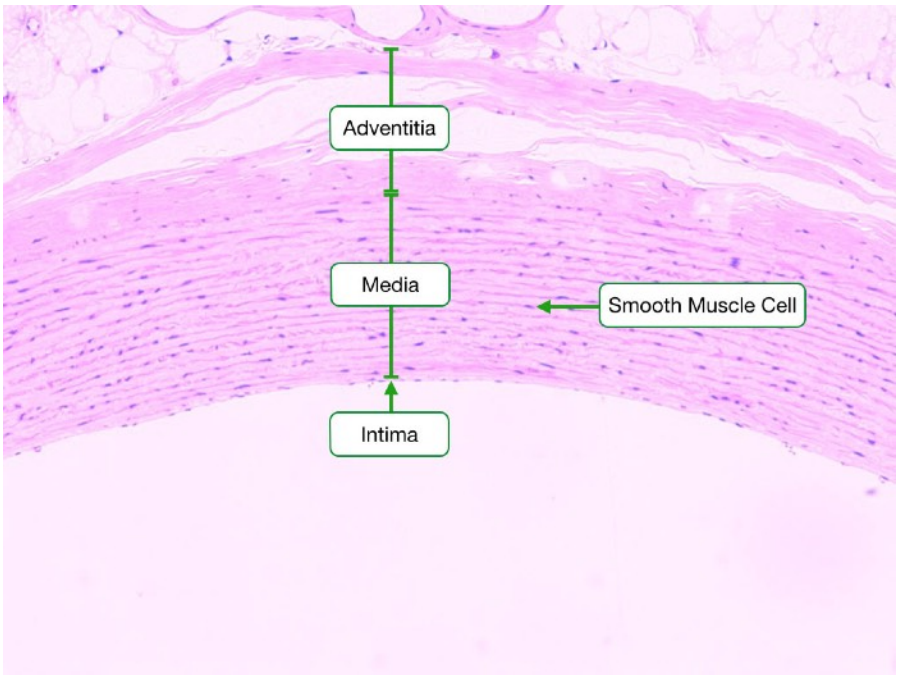
In many tissues and organs, arteries and venules often run together. This image compares the structure of a venule and small artery and highlights their differences. The lumens of the vessels are similar in size, but the artery has a thicker medial layer with more smooth muscle. Venules, with thinner walls, are more compliant and capable of holding more blood. Consequently, arteries tend to maintain their round shape better than veins in histological sections. Veins also contain valves to prevent back flow of blood but these are infrequent and are not reliably seen in histological samples.



Venules have thinner medial layers than arteries and tend to lose their shape.

Vena Cava

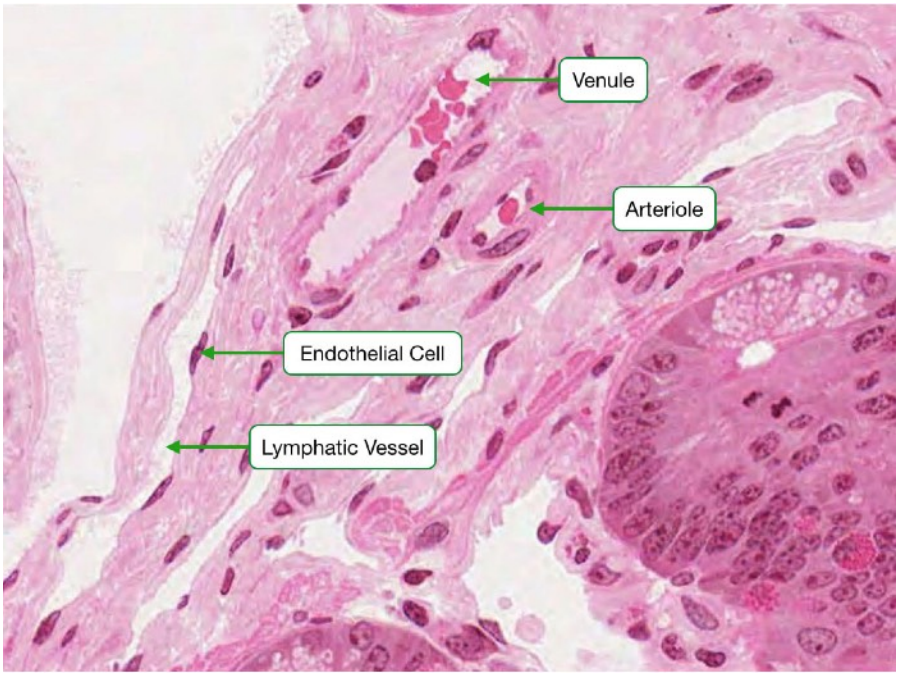
This image shows the wall of the vena cava, which is the largest vein in the body. Note the relatively thin media compared to the aorta. The media layer contains primarily smooth muscle cells and collagen with very few elastic fibers.



The vena cava has a thinner media than the aorta with mostly smooth muscle cells and collagen.

Lymphatic Vessel

Lymphatic vessels are responsible for draining interstitial fluid and returning it to the bloodstream. These vessels are lined by endothelial cells and have a very thin layer of smooth muscle. Like veins, lymphatic vessels have valves that prevent back flow. Lymphatic vessels notably lack red blood cells, which help distinguish them from veins. The lymphatic system also plays an important role in generating immune responses.

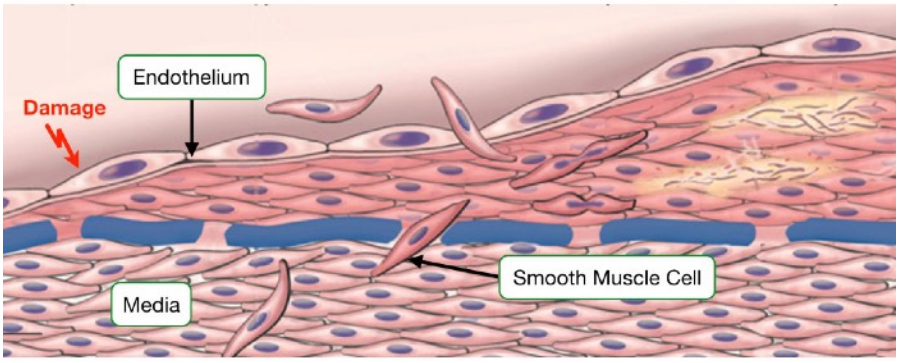


Lymphatic vessels have thin walls and lack red blood cells.

Response to Damage

The endothelium is prone to damage due to the shear forces generated by flowing blood. Like epithelia, endothelium have mechanisms to repair damage and regenerate endothelia cells.

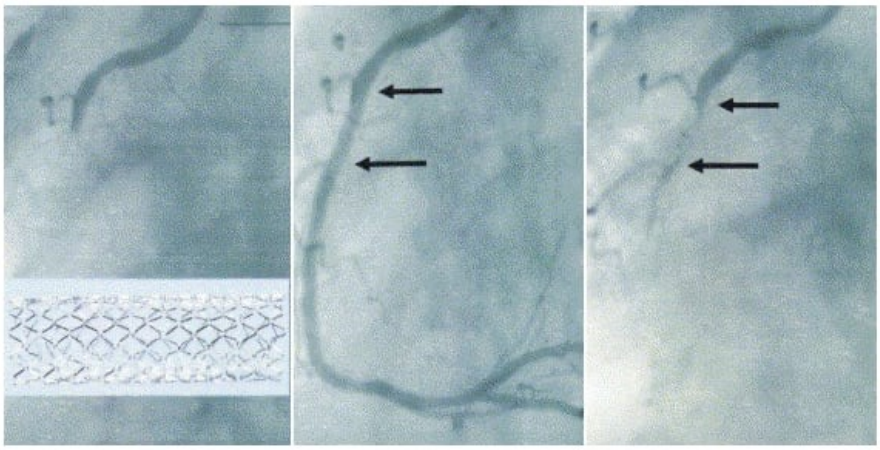
Surprisingly, it appears that the smooth muscle cells in the media play a prominent role repairing the endothelium. Damage to endothelial layer stimulates recruitment of smooth muscle cell progenitors. These cells synthesize connective tissue molecules such as collagen to help repair wound. These progenitors come from the adventia and media but also the blood. The progenitors are capable of dividing and one consequence is proliferation of smooth muscle in the intimal layer which causes bulging of intima and occlusion of the vessel.



Damage recruits smooth muscle cell progenitors to the intima.

Angioplasty is one common treatment for occluded arteries. Stents are inserted to keep the artery open. The procedure, however, damages the endothelial layer of the vessel and often trigger

proliferation of smooth muscle cells in the intima. Over time the growth of smooth muscle cells occludes the artery despite the presence of the stent. To prevent the growth of smooth muscle cells, stents were designed to release chemicals that inhibit the proliferation of smooth muscle cells.

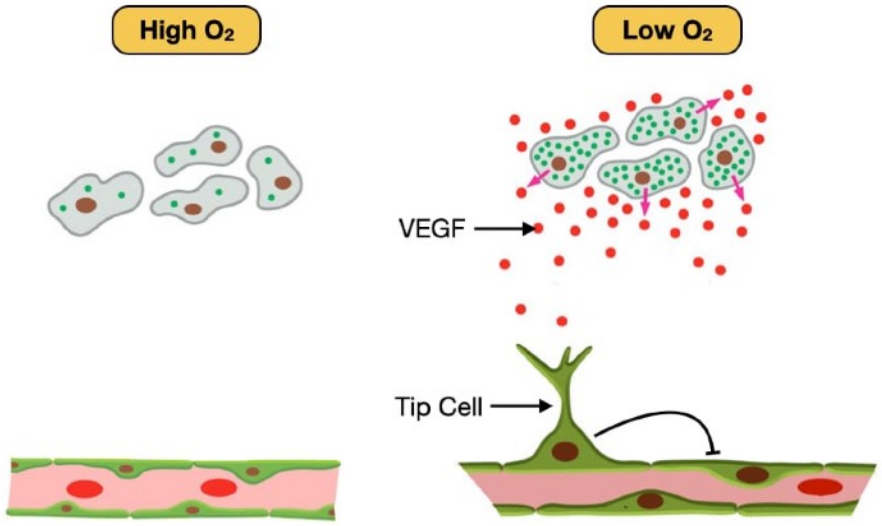


Smooth muscle proliferation can lead to occlusion of arteries after insertion of stents.

Angiogenesis

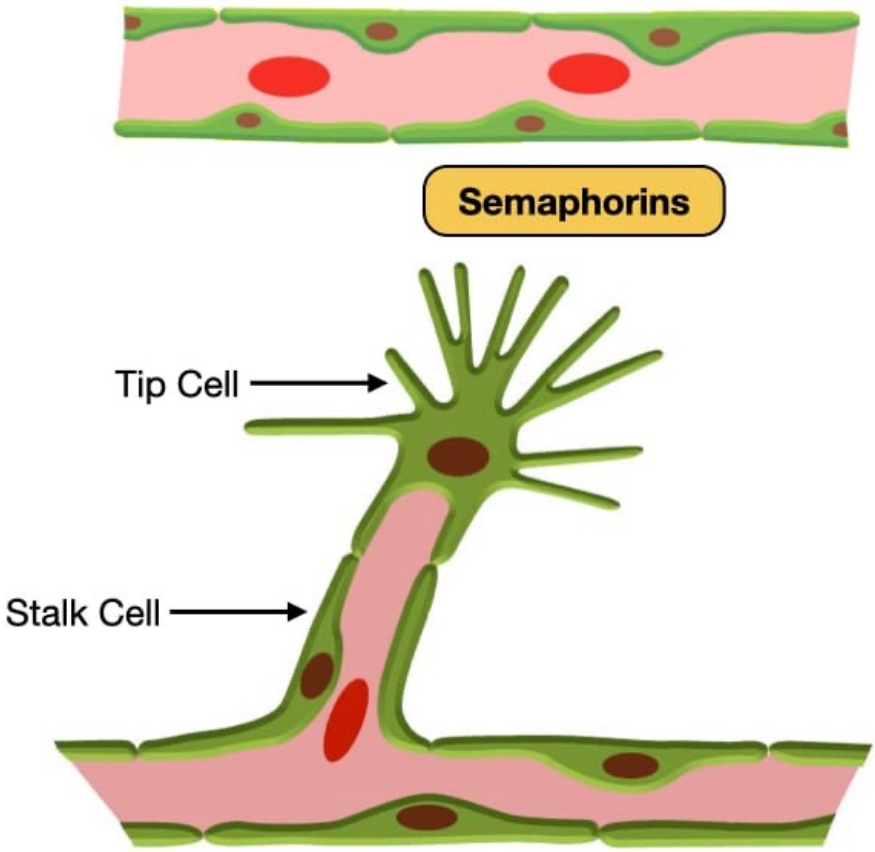
Angiogenesis is the growth of new blood vessels and is seen during hypoxia or low oxygen conditions and wound repair.

Endothelial cells are also responsible for initiating growth of new vessels. In response to low oxygen or wounding, cells within a tissue express several proteins, the most prominent being vascular-endothelial growth factor (VEGF), that trigger growth of new vessels. VEGF causes certain endothelial cells, called tip cells, to undergo several radical changes. First, the tip cells reverse polarity, so whereas the apical surface normally faces the lumen, VEGF causes the apical surface to face the surrounding tissue. Second, the junctional complexes between tip cells and neighboring endothelial cells are changed. Finally, the tip cell invades the surrounding tissue by extending filopodia. Only tip cells respond to VEGF because if all cells responded, the entire vessels would degenerate. Tip cells prevent neighboring endothelial cells from responding to VEGF.



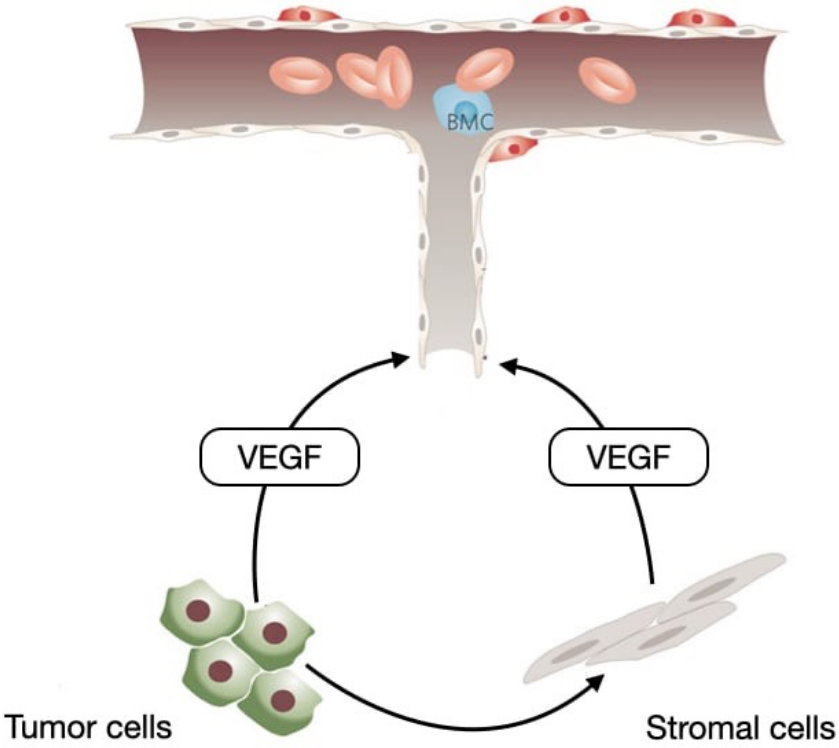
VEGF triggers morphological changes in endothelial tip cells.

The crawling of the tip cell is guided by a set of signaling molecules called semaphorins that are expressed by the surrounding tissue. Semaphorins also guide the growth of axon during neural development. Following the tip cell are specialized endothelial cells called stalk cells that can divide to produce more endothelial cells and have the ability to form the lumen of the new vessel behind the tip cell. Eventually the tip cell will reach an existing vessels and integrate into the vessel to form a conduit between vessels.



Tip cells follow guidance cues to connect to existing vessels.

VEGF also appears to play an important role in the growth of tumors. Due to high cell mass, tumors are hypoxic environments. Tumors activate production of VEGF to recruit blood vessels to the tumor. Anti-VEGF antibodies (Avastin) are a treatment to slow growth of tumors.



Tumor cells initiate angiogenesis to increase their blood supply.