The Functional Importance of Vascular Endothelium for Inhibiting Coagulation of Blood Injected into the Femoral Artery Using the Claudere Vascular Femoral Artery Sealant

Vascular endothelium, the continuous, single-cell thick lining of the cardiovascular system, constitutes "Nature's Blood Compatible Container." As long as blood stays within this container, it remains fluid and can perform its essential transport and communication functions unimpeded. However, simple contact of blood with a nonendothelial surface is sufficient to trigger platelet activation and the coagulation cascade. The importance of the vascular lining for the maintenance of blood fluidity was appreciated as early as 1856 by Rudolph Virchow and formed part of his classic triad of predisposing factors for a pathologic thrombosis. Back in the time of Virchow, this "nonthrombogenic" nature of the vascular lining was believed to be more passive than active. However, more recently, this property was considered to be more active. Endothelial cells synthesize prostacyclin (the most potent natural occurring inhibitor of platelet aggregation). In addition to the major influence on platelet function, endothelium also appears to be a pivotal element in the coagulation and fibrinolytic systems. Several "natural anticoagulant mechanisms" are in effect to maintain blood fluidity. These mechanisms include the protein C-Thrombomodulin mechanism and the tissue plasminogen activator mechanism.

The Claudere Vascular Femoral Artery Sealant is unique in that it only uses the patient's own blood to stop bleeding from the wound site. The blood is activated by contact activation with a foreign surface, shear activation, formation of low levels of thrombin, and removal of heparin from the blood sample. In the event that the activated blood sample (approximately 5 ml) is injected directly into the femoral artery instead of into the track to which it is intended to close. "Nature's Blood Compatible Container" will serve to maintain the blood on the fluid phase and prevent fibrin formation and clot deposition.

The following information is intended to serve as background description of the natural events that take advantage of vascular biology and provide a unique safety feature of Claudere Vascular Femoral Artery Sealant.

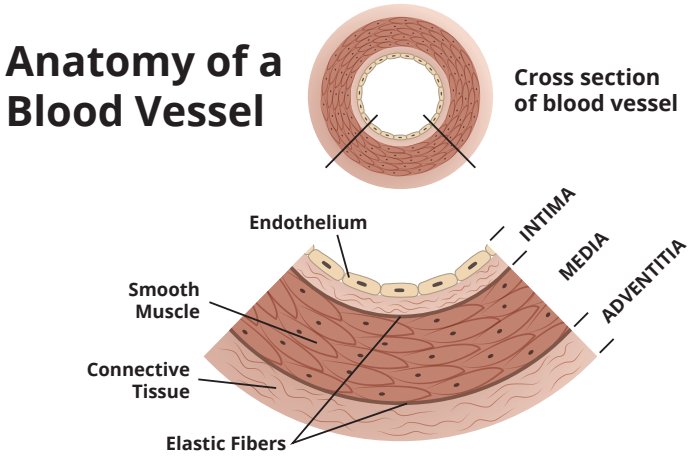
ANATOMY OF BLOOD VESSELS

Before addressing the biochemistry of the vascular endothelium, a brief anatomy of the blood vessels follows:

The blood vessels are a closed system of conduits that carry blood from the heart to the tissues and back to the heart. Blood vessels are classified as arteries, capillaries, and veins—each of which is distinguished by size, thickness and function.

1. **Arteries** –Thick walled vessels designed to withstand the higher pressures of the systemic circulation. The walls of arteries are composed of three concentric layers:
   a. **Intima** – The innermost layer adjacent to the lumen composed of a single layer of endothelial cells along with their basement membranes and underlying connective tissue.
   b. **Media** – Consists primarily of smooth muscle.

c. **Adventitia** – Composed of connective tissue. The adventitia of larger arteries also contain the vasa vasorum – small blood vessels which supply nourishment to the outer portion of the media and adventitia.



A band of tissue composed of **elastin fibers** separates the intima from the media, and the media from the adventitia. The aorta and larger arteries (innominate, subclavian, common carotid, iliac, and pulmonary arteries) contain an abundant amount of elastic tissue. These vessels are stretched by the force of cardiac ejection during systole and then recoil during diastole. This boosts the forward motion of blood through the smaller branches of the circulation and is responsible for diastolic blood pressure.

Arterioles are the smallest branches of arteries. They, along with medium sized arteries such as the coronary and renal arteries, contain less elastic tissue, but proportionally more smooth muscle. The smooth muscle is richly innervated by noradrenergic nerve fibers of the autonomic nervous system, which cause vasoconstriction (the sympathetic nerves supplying the arterioles of skeletal muscles are cholinergic and cause vasodilatation). By varying the diameter of arterioles, the circulation regulates systemic arterial blood pressure and the flow of blood to tissues. Because the resistance to blood flow is inversely proportional to the fourth power of the radius of a blood vessel, small changes in the size of the lumen of arterioles can have a profound effect on blood flow. For example, reducing the radius of a coronary artery by one-half increases resistance to flow by a factor of 16 and greatly impairs blood flow to the myocardium.

2. **Capillaries** – These vessels are approximately the diameter of a red blood cell (7-8 um), and consist of only a single layer of endothelial cells and basement membrane. There is no smooth muscle or connective tissue in capillaries. Arterioles drain into capillaries, which, in turn, drain into venules. The upstream (arteriole) openings of capillaries are surrounded by smooth muscle **precapillary sphincters**. Capillaries are the site of exchange of nutrients, oxygen, CO2, and other metabolic substrates between the circulation and various tissues of the body.

3. **Venules and Veins** – Venules and veins receive flow from capillaries and drain into the superior and inferior vena cava which, in turn, drain into the right atrium of the heart. Venules are similar to capillaries except that they have a connective tissue sheath. Larger veins are thin walled vessels that contain only a modest amount of smooth muscle and connective tissue. The intima of veins in the limbs is folded at intervals to form the **venous valves** that prevent back flow of blood.

The walls of veins are easily distended and can expand to hold more blood without much increase in intravascular pressure. For this reason veins are sometimes referred to as capacitance vessels. The smooth muscle of veins is innervated by noradrenergic nerves. Variation in the contraction state of this smooth muscle (vasoconstriction, vasodilation) can control how much blood returns to the right side of the heart.

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4. **The functional importance of vascular endothelium**: How the vascular serves Nature's Blood-Compatible Container.

a. Endothelial cells form a single layer that lines the entire vascular system. They serve as a semipermeable membrane regulating the transfer of small and large molecules into the arterial wall, and through the walls of capillaries and venules.

b. Endothelial cells also synthesize and secrete a number of important biological molecules that play a role in modulating blood flow, inflammation, and cell growth.

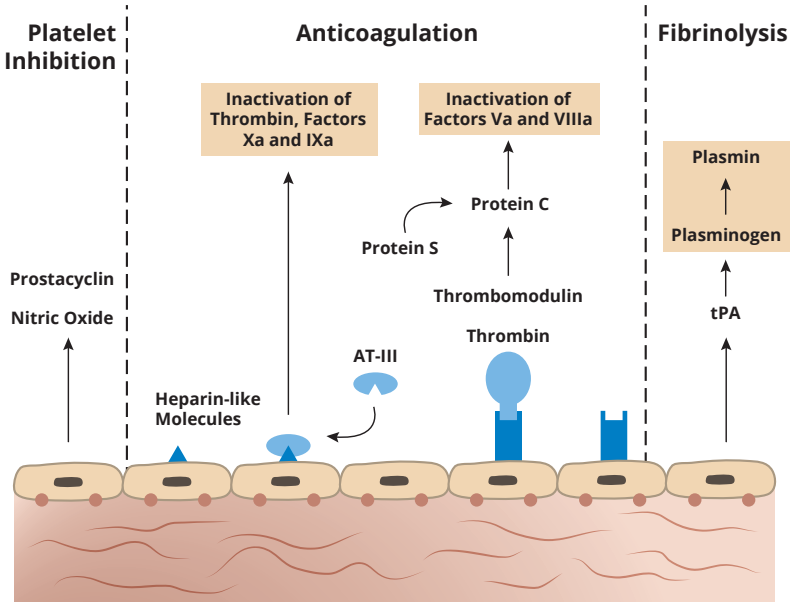
- **Prostacyclin** (a prostaglandin) and **nitric oxide,** produced by endothelial cells, cause relaxation of vascular smooth muscle and help dilate vessels. Another class of compounds called **endothelins** are potent vasoconstrictors. The relative amounts of vasodilators and vasoconstrictors produced by endothelial cells help control local blood flow through various tissues.

- Adhesion molecules on the surface of injured endothelium allow white blood cells to leave the circulation and respond to tissue injury and foreign invaders.

- A number of cytokines elaborated by endothelial cells regulate the proliferation of vascular smooth muscle and promotes the growth of new blood vessels.

c. Intact endothelium protects against inappropriate activation of platelets and inhibits the formation of intravascular blood clots (thrombosis). In addition to masking platelets and plasma proteins from tissue factor and subendothelial collagen, endothelial cells also secrete a number of anti-thrombotic molecules.

The following schematic diagrams the events that occur in concert to maintain blood fluidity.



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- The vasodilators prostacyclin and nitric oxide inhibit platelet aggregation.

- **Heparin-like molecules** on the surface of endothelial cells combine with the plasma protein antithrombin III (AT-III) to inactivate thrombin and factors Xa and 1Xa. Similarly, another cell surface molecule, **thrombomodulin,** binds thrombin so that it cannot convert fibrinogen to fibrin. The thrombomodulin-thrombin complex also activates two other naturally occurring anticoagulants, Protein C and Protein S, which degrade Factors Va and VIIIa.

- Endothelial cells secrete **tissue plasminogen activator (tPA),** which activates plasmin and the fibrinolytic system.

1. **Thrombosis and Thromboembolism**

   a. **Definitions** – A t**hrombus** is a solid mass composed of platelets, fibrin and entrapped red blood cells that forms in the circulation. By definition, a thrombus is *attached to vascular endothelium* (or to the endocardial lining of the heart). Thrombosis can occur in the arterial tree, the heart, or within the venous system. Common locations for arterial thrombi include the cerebral, coronary, mesenteric, and renal arteries. Thrombi can also develop on the wall of a heart chamber **(mural thrombus**). In the venous circulation, thrombi most commonly form in the large, deep veins of the lower extremities (**deep venous thrombosis).**

   A **thromboembolus** is a fragment of a thrombus that *detaches and is carried downstream to lodge in a smaller vessel.*

OTHER TYPES OF CLINICALLY SIGNIFICANT EMBOLI…(NOT FACTORS INVOLVED WITH THE CLAUDERE VASCULAR SYSTEM)

- **Air embolism** – Introduction of large amounts of air (usually greater than 100 ml) can physically obstruct the flow of blood to the right side of the heart, the pulmonary circulation, and the brain. This most commonly occurs in the setting of neck wounds, thoracentesis, punctures of large veins, and with hemodialysis. Air embolism is part of the clinical picture of decompression sickness seen in deep sea divers.

- **Amniotic fluid embolism** – This disorder occurs during labor when amniotic fluid and fetal cells enter the maternal circulation through open uterine veins. Because amniotic fluid has thrombogenic properties, pulmonary emboli or even DIC can result.

- **Fat (bone marrow) embolism** – Fractures of long bones, sternum, or ribs may release hematopoietic cells and fat into the circulation. Embolization to lungs or brain may occur.

WHAT CAUSES THROMBOSIS? – THE PATHOGENESIS OF THROMBOSIS INVOLVES THREE FACTORS:

- **Endothelial injury** – Endothelial injury exposes platelets and plasma coagulation factors to sub endothelial collagen and tissue factor activating primary and secondary hemostasis. This process is aided by the loss of normal endothelial coagulation inhibitors from injured endothelium.

Common mechanisms of endothelial/endocardial injury include myocardial infarction, endocarditis, vasculitis, ulceration or rupture of atheromatous plaques, traumatic vascular injury (e.g. burns). Ionizing radiation, certain bacterial toxins, toxins in cigarette smoke, hypercholesterolemia, and elevated levels of homocysteine can also injure vascular endothelium.

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- **Alteration to normal blood flow** – Normal blood flow keeps platelets in the middle of the blood stream and away from the vessel wall where they can be activated. Normal blood flow also dilutes and/or carries away any thrombin or activated plasma coagulation factors that may form inadvertently in the circulation. Slowing of blood flow (stasis) or turbulence in the circulation can bring platelets into contact with the endothelium leading to the activation of primary hemostasis. Turbulent blood flow may also injure endothelial cells.

  Turbulence and stasis occur in dilated vessels (aneurysms, venous varicosities), stenotic blood vessels or heart valves, and around atherosclerotic plaques. The noncontractile myocardium of a myocardial infarction can produce pockets of stasis in the ventricles, promoting the formation of mural thrombi. Similarly, thrombi can form in a dilated or fibrillating atrium.

  Although less common, hyperviscosity of the blood (polycythemia vera, sickle cell anemia, plasma cell malignancies) may also slow blood flow enough to cause thrombosis.

- **Increased coagulability of blood** – Thrombosis may occur when the blood becomes "hypercoagulable" due to an inherited or acquired deficiency of normal plasma coagulation inhibitors such as AT-III, Protein C, or Protein S.

  The most common inherited hypercoagulable state involves a mutation in Factor V (Factor V Leiden) that prevents it from being inactivated by Protein C. Homocysteinuria is associated with an increased risk for arterial and venous thrombosis. Increased levels of estrogen (pregnancy and birth control pills) cause a decrease in Protein C and Protein S. Many cancers are associated with the elaboration of pro-coagulants that increase the risk for thrombosis. An important immune-mediated cause of thrombosis risk is the antiphospholipid syndrome. In this condition, high levels of autoantibodies directed against phospholipids and certain proteins induce a hypercoagulable state (exact mechanism unclear).

WHAT HAPPENS TO A THROMBOSIS AFTER IT FORMS?

- The fibrinolytic system may completely degrade the clot, allowing blood flow to return to normal.

- The thrombus may "propagate" – accumulate more fibrin and platelets and grow along the course of the vessel.

- The thrombus may become fibrotic and be incorporated into the wall of the blood vessel. In some cases new blood vessels may grow into the fibrotic thrombus and establish partial but reduced blood flow (recanalization).

- Thrombi may dislodge and travel to other sites in the circulation (thromboembolus).

What are the clinical consequences of thrombosis?

– The major clinical consequences of thrombus formation are narrowing and occlusion of blood vessels, or the generation of an embolus. Both of these can lead to tissue ischemia and infarction.

- **Thrombosis in the arterial system** – Myocardial infarction, cerebral infarction (stroke), mesenteric thrombosis, peripheral vascular disease (intermittent claudication, gangrene).

- **Thrombosis in the heart** – Formulation of a **mural thrombus** – i.e., a thrombus attached to the endocardial surface of a heart chamber. This occurs most frequently in the setting of myocardial infarction, atrial fibrillation, and cardiomyopathy. Thrombi may also develop on cardiac valves (usually mitral or aortic) that are damaged by bacterial infection (bacterial endocarditis). Mural thrombi may dislodge and produce arterial emboli.

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WHITE PAPER

EXPERIMENTAL RESULTS OF 5CC of BLOOD INJECTED INTO THE FEMORAL ARTERY AFTER PASSING OVER THE CLAUDERE VASCULAR DEVICE

During preclinical work, extensive safety testing was performed per FDA protocols to establish the safety of the Claudere Vascular system in the event blood treated in the Claudere Vascular reaction cartridge was inadvertently introduced into general circulation. Testing included injecting ten times the normal amount of treated blood into renal and femoral arteries and then looking for evidence of fibrin organization or thrombus formation using full body digital subtraction angiography and cine angiography as well as biochemical testing and necropsy. No evidence of clot formation was ever detected by any method. A full report of the testing protocols and results is available upon request.

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