3.6.4

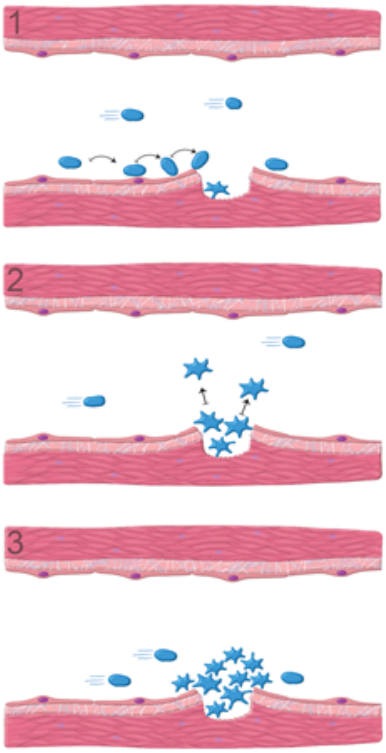
Hemostasis

Hemostasis, or the cessation of bleeding, is critical to the survival of the human organism. If the mechanisms of hemostasis were completely taken away, a mere paper cut could ultimately lead an individual to bleed to death. Luckily, the mechanisms of hemostasis function properly for most individuals. Hemostasis can be divided into three steps: vascular spasm, development of a platelet plug, and blood clot formation.

Vascular Spasm

Upon incurring some type of trauma, blood flow through damaged vessels is immediately limited through a process known as **vascular spasm.** During a vascular spasm, pain can cause reflexes in the nervous system to stimulate smooth muscle contraction around the blood vessel. As the smooth muscle of the impaired vessels contract, the diameter of the vessels decrease. In some instances, the blood vessel can be entirely occluded by a vascular spasm. However, this occlusion is short lived and the smooth muscle will eventually relax and then bleeding out of the damaged vessel can greatly increase. As effective as a vascular spasm is, it is temporary and cannot establish hemostasis alone.

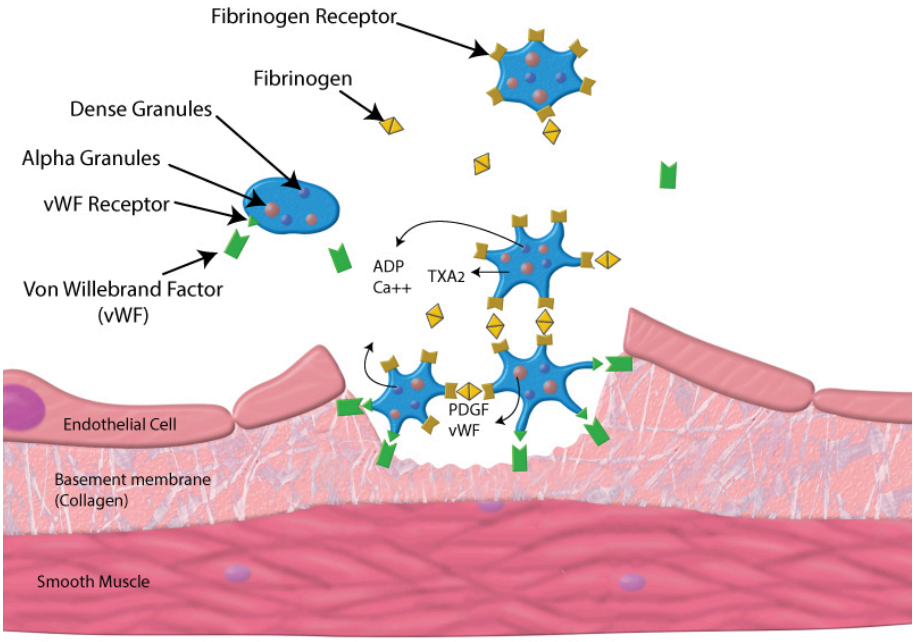
Platelet Plug



Formation of Platelet Plug

Image drawn by BYU-Idaho student Nate Shoemaker Spring 2016

The image above shows a blood vessel in longitudinal section. Number one shows how platelets begin to converge, slow down and roll along the vessel wall near the damaged area. Platelets begin to attach to the collagen in the damaged area and this activates them. Activated platelets experience a physical shape change and are able to attract other platelets to attach to them and to any other exposed collagen. This is called a platelet plug. The image below shows a close up of the platelet plug and more detail involved in the chemical messengers that facilitate a successful platelet plug.



PDGF = Platelet Derived Growth FactorHistamine; TXA2= Thromboxane (also called PAF or Platelet Activating Factor); vWF= Von Willebrand Factor

Platelet Activation

Image drawn by BYU-Idaho student Nate Shoemaker Spring 2016. Description below by T. Orton Winter 2017

Platelet Plug Formation in Injured Blood Vessel

1. Vascular spasm to constrict blood flow to area
2. vonWillebrand Factor (vWf) secreted by endothelial cells
3. vWF bind Platelets to Collagen in vessel wall
4. Bound Platelets are activated:
   - **Change Shape.**
   - **"Alpha" granules** in Platelets release more vWF, Fibrinogen & Platelet Derived Growth Factor (PDGF)
   - **"Dense" granules** in Platelets release Adenosine Diphosphate (ADP), Calcium, & Serotonin
   - **Thromboxane (TXA2)** released from membrane
   - **Receptors:** vWf Receptors and Fibrinogen receptors appear on the surface of activated Platelets
5. Thromboxane and ADP activate more Platelets
6. Fibrinogen binds to Fibrinogen Receptors on activated platelets and link Platelets to Platelets.
7. Clotting Coagulation Cascade activated. Clotting factors lead to Factor X converting Prothrombin to Thrombin
8. Thrombin converts Fibrinogen into **Fibrin to** create a Fibrin mesh to strengthen plug. Red and white blood cells are caught in fibrin mesh.
9. Actin and Myosin in platelets contract to seal off the wound

When blood vessels suffer damage, a protein secreted by endothelial cells known as **von Willebrand factor (vWf)** binds to exposed collagen within the vessel wall. Circulating platelets in turn bind to the collagen bound molecules of von Willebrand factor and anchor themselves to the damaged area. This interaction also causes a platelet activation which increases the likelihood that more platelets will accumulate.

Following platelet adhesion, the anchored platelets begin to secrete a variety of chemical compounds from granules called "alpha" and "dense" granules. Alpha granules release additional von Willebrand factor and platelet derived growth factors (PDGF). vWF assists with further platelet adherence and activation. PDGF facilitates a variety of functions that assist in the long-term wound healing of tissue damage. Dense granules release adenosine diphosphate (ADP). ADP and thromboxane (also called TXA2 and released from the platelet cytoplasm) bind to surface receptors on additional circulating platelets and promote further activation. These newly activated platelets, in turn, activate additional platelets, establishing a chemical cascade.

Platelet activation also results in the expression of membrane receptors called fibrinogen receptors. These receptors bind a plasma protein known as **fibrinogen**. Like von Willebrand factor, fibrinogen serves as a type of linking molecule. However, whereas von Willebrand factor links collagen to platelets, fibrinogen links multiple platelets together. Generally, enough platelets link up that they span across and "plug" the opening in a damaged vessel. Platelets are rich in the proteins actin and myosin which allows for contraction which forms a more compact platelet plug. This is called Platelet Aggregation.

Platelet Plug Regulation

Unless you have a clotting disorder (like hemophilia), your blood will spontaneously form a clot in order to close an open wound. But what is it about the wound that induces clot formation? Equally important, how does your body prevent clot formation when there is no wound? In order to understand hemostasis (the “stopping” of blood flow), it is important to first understand the chemical signals that block hemostasis under normal conditions (because spontaneous clot formation is very painful and can be lethal). Spontaneous clot formation is primarily prevented by the active inhibition of platelets, and the most important cell type to regulate platelets is the endothelial cell.

Healthy, intact endothelial cells block spontaneous platelet activation using three primary mechanisms. However, all three mechanisms share a common goal: reduce Ca^{2+} levels within the platelet. Acting as a second messenger, increased Ca^{2+} within platelets will lead to the exocytosis of platelet granules. Granule release is synonymous with platelet activation. Thus, by reducing Ca^{2+} , platelets are maintained in an inactive state. The first approach used by endothelial cells to inhibit platelet activation is the production and secretion of nitric oxide (NO). Secreted NO enters platelets where it activates the enzyme guanylate cyclase, which then stimulates the formation of cGMP (cyclic guanosine mono-phosphate). cGMP then blocks the function of phospholipase C. In the absence of NO, phospholipase C cleaves the membrane phospholipid PIP2, which leads to the production of inositol triphosphate (IP3). IP3 then binds to and opens Ca^{2+} channels on the surface of the endoplasmic reticulum, causing Ca^{2+} to flood the cell. And remember – intracellular Ca^{2+} causes exocytosis (granule release). Thus, by blocking the release of Ca^{2+} from the ER, NO from intact endothelial cells inhibits platelet granule release.

Another mechanism used by endothelial cells to inhibit platelet activation is the production and secretion of a signaling molecule called **prostacyclin**. Prostacyclin is the ligand for a G-protein coupled receptor (GPCR) on the surface of the platelet. Activation of this GPCR by prostacyclin leads to the production of cAMP. cAMP then activates a Ca^{2+} pump on the surface of the platelet, which actively pumps Ca^{2+} out of the platelet. Thus, increased Ca^{2+} efflux reduces Ca^{2+} within the platelets, which inhibits granule release.

Third, endothelial cells express an ADPase on the surface of the cell. This ADPase is an enzyme that metabolizes ADP to AMP (adenosine mono-phosphate). ADP is a potent platelet activator and works by binding to ADP receptors on the surface of the platelet. Activation of this receptor (P2Y12) directly inhibits the effects of prostacyclin (described in the previous paragraph). As such, P2Y12 activation causes Ca^{2+} to build up inside the cell, resulting in granule release.

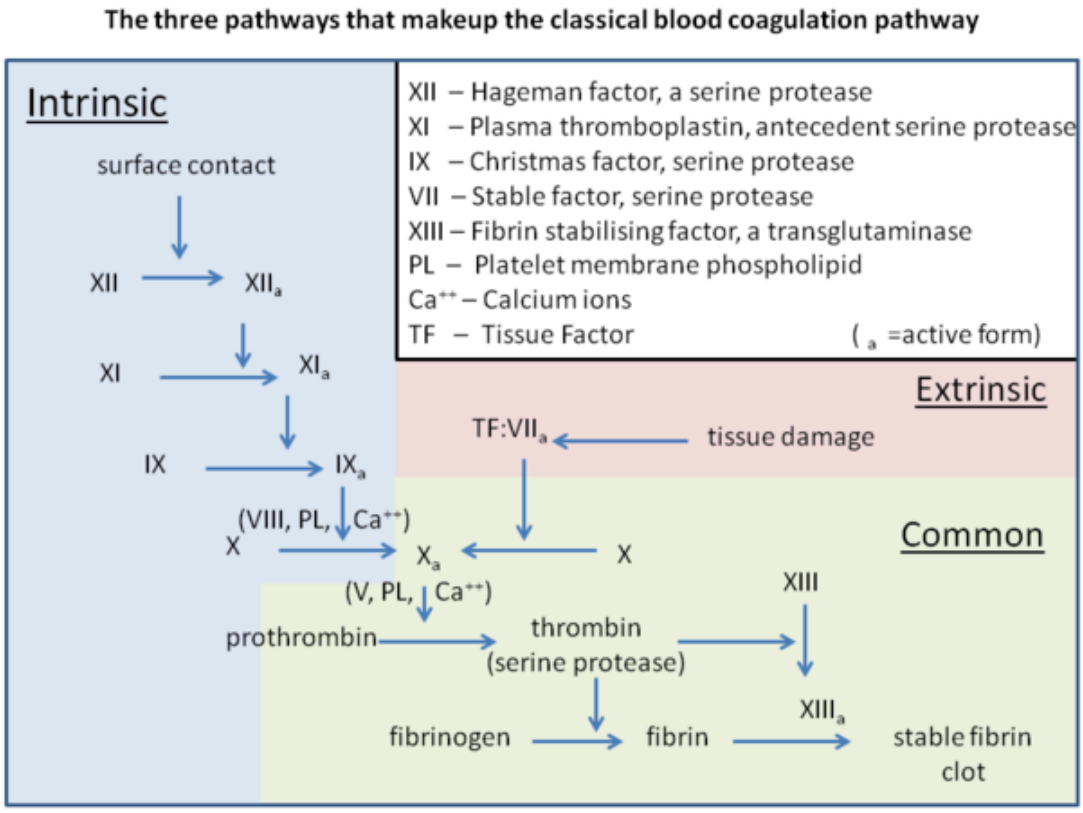
The endothelial ADPase inhibits P2Y12 activation by eliminating its ligand, ADP.

Interestingly, you have likely seen TV commercials for the drug Plavix (clopridogel bisulfate). Plavix is often prescribed to patients following a heart attack or stroke to help prevent clot formation. Plavix is a P2Y12 antagonist. As you can see, each strategy regulates the buildup of Ca^{2+} within platelets. However, following injury, these inhibitory mechanisms are overcome, resulting in platelet granule release and the initiation of platelet plug formation. It is also interesting to note that one of the most potent stimulators to increase endothelial cell production of NO, ADPase and Prostacyclin is a protein called Thrombin. As you continue to read about hemostasis, try to appreciate how thrombin becomes an integral part of a negative feedback loop that can help regulate the positive feedback initiated during clotting.

Clotting (Coagulation) Cascade

Simultaneously with the mechanisms of hemostasis listed above, a process known as **coagulation** occurs. Coagulation requires an array of proteins known as **coagulation factors** or **clotting factors** which constantly circulate within the plasma in an inactive state. These factors are typically represented by Roman numerals which indicate the order in which they were identified and offer no explanation as to their function. Upon encountering vessel damage, certain of these factors become activated and initiate complex chemical cascades which ultimately lead to the formation of a blood clot. This activation may occur through either an **intrinsic pathway** (within the bloodstream) or an **extrinsic pathway** (outside of the blood stream). Both pathways ultimately lead to a **common pathway** through which a blood clot forms.

The chart below shows the cascade of clotting factor activation. The pathway has two entry points to begin the cascade, the "intrinsic" and "extrinsic" pathways. The common pathway begins with the activation of clotting factor X, which is also called Stuart Factor".



Classical Blood Coagulation Pathway

Intrinsic Pathway

When blood vessels incur damage, collagen within the vessel walls is exposed to the circulating bloodstream. Contact with collagen reacts with factor XII which in turn activates factor XI which sequentially initiates factor IX. Factor IX recruits factor VIII, platelet phospholipids, and Ca^{2+} ion cofactors to form a complex that activates factor X, thereby initiating the common pathway.

Extrinsic Pathway

Unlike the intrinsic pathway which is activated by exposed collagen, the extrinsic pathway is activated by chemical signals released by damaged tissues external to the bloodstream. Damage to these tissues destroys cellular plasma membranes yielding a collection of phospholipids and an integral receptor protein known as **tissue thromboplastin** (alternatively known as factor III or tissue factor). As blood rushes into these damaged tissues, circulating molecules of factor VII associate with the released combination of factor III molecules and phospholipids to form an enzymatic complex. Ca^{2+}serves as a required cofactor for the formation of this complex. This complicated enzymatic complex reacts with and activates factor X and the common pathway is again initiated. This factor III, VII, phospholipid and calcium complex are also capable of activating factor IX within the intrinsic pathway, indicating a one-way connection between the two pathways.

Common Pathway

Upon activation, factor X joins with factor V, Ca^{2+}, and platelet surface phospholipids to form a **prothrombin activating complex**. This complex target **prothrombin** (factor II) and converts it to a molecule known as thrombin. Thrombin is an important enzyme which converts a protein known as **fibrinogen** into a fibrous clot forming protein known as **fibrin**.

Fibrin plays a critical role in the formation of a clot by forming a dense, fibrous weave against which blood "congeals" into a thicker, gel-like clot capable of clogging damaged areas of vessels.

The activation of thrombin initiates a positive feedback mechanism, as thrombin is capable of activating numerous factors within the coagulation pathways. Consequently, thrombin exerts a stimulatory effect on its own production. Furthermore, thrombin also activates factor XIII which stabilizes the clot by catalyzing the formation of covalent bonds between fibrin strands. Finally, thrombin initiates additional platelet activation. Thus, the thrombin positive feedback mechanism stimulates further platelet plug proliferation in addition to its coagulation effects.

Clotting Factors: Number, Name and Description

Factor Number	Name	Description
I	Fibrinogen	Plasma protein produced by the liver. Fibrinogen can form bridges between activated platelets, but is more known for its major function as a precursor to fibrin.
II	Prothrombin	Plasma protein produced by the liver. It is activated by a complex of factor X and V to become thrombin. Thrombin is important because it converts fibrinogen to fibrin.
III	Tissue Thromboplastin (Tissue Factor)	This is an integral membrane protein produced by cells outside of the vascular conduits of the circulatory system. It is necessary for the first step of the extrinsic pathway. This protein is a receptor for a plasma protein called factor VII. When exposed to factor VII, a large enzyme complex is formed that can activate factor X (the common pathway).
IV	Calcium ions	Required as a cofactor for many of the enzymatic reactions that take place in the clotting cascade.
V	Proaccelerin (Labile Factor)	A plasma protein produced by the liver. Factor V is a cofactor that can associate with Factor X and accelerate the conversion of Prothrombin to Thrombin.
VI		Now known to be just activated Factor V, so a distinct factor VI is no longer considered to exist.
VII	Serum Prothrombin Conversion Accelerator (stable factor, proconvertin)	Plasma protein synthesized in the liver. Important in the extrinsic pathway as it helps form an enzyme complex with tissue thromboplastin.
VIII	Antihemophilic factor (antihemophilic globulin)	A plasma protein that is synthesized in the liver as well. It is a cofactor with factor IX to activate factor X of the common pathway. Factor VIII is an important component of the intrinsic pathway.
IX	Plasma Thromboplastin Component (Christmas Factor)	Another plasma protein synthesized by the liver. It is important for the activation of factor X. This factor is another component of the intrinsic pathway.
X	Stuart Factor (Stuart - Prower factor)	Synthesized in the liver and is found as a plasma protein that when activated can from a complex with Factor V, phospholipids and calcium. This complex is

Clotting Factors: Number, Name and Description

		the first step of the common pathway and has the job of activating prothrombin to thrombin.
XI	Plasma Thromboplastin Antecedent	Another plasma protein synthesized in the liver. It is an important intermediary in the intrinsic pathway as it is responsible for activating factor IX.
XII	Hageman Factor	Yet another plasma protein coming from the liver that travels in the blood. It is activated by contact with polyanions (molecules with a lot of negative charges). If a blood vessel has damage to the endothelium, the collagen comes into contact with factor XII. The collagen proteins have multiple negative charges throughout the macromolecule and this is enough to activate factor XII. Factor XII activation is the first step of the intrinsic pathway.
XIII	Fibrin Stabilizing Factor	A plasma protein synthesized in the bone marrow and possibly liver. This protein, once activated, can covalently bond with fibrin in a way that "cross links" are formed. This makes the fibrin network insoluble and more stable.

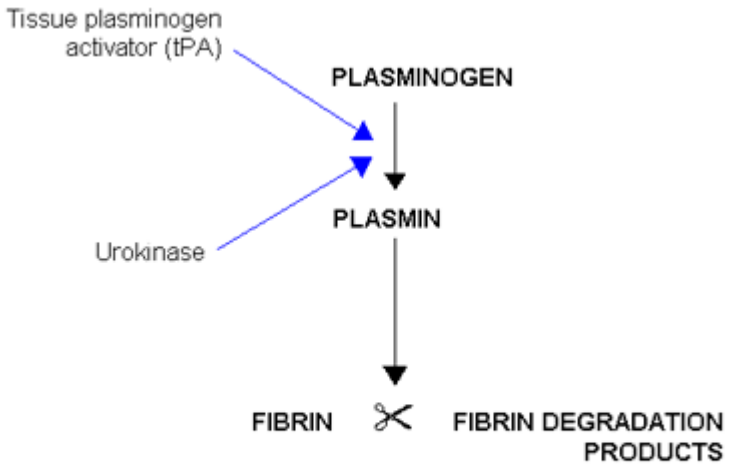
Thrombus vs Embolus

A blood clot which will include platelets and fibrin is also called a **thrombus** if it is anchored to the vessel wall where it was created. If this thrombus breaks away from the vessel wall and begins to circulate in the vascular system, it is called an **Embolus** or an Embolism. An embolus can be very dangerous because it may get stuck in a small blood vessel and block blood flow from reaching cells further downstream. Cells die without constant blood flow reaching them. If an Embolus blocks enough blood flow to cells in the heart or the brain, it can quickly become lethal.

Clot Retraction and Fibrinolysis

Keep in mind that blood clots form upon and in conjunction with a preexisting platelet plug. Upon clot formation, a process known as **clot retraction** occurs. During clot retraction, actin and myosin contained within platelets of the platelet plug begin to contract. As these proteins contract, the web of platelets connected by molecules of fibrinogen begins to retract and condense. This, in turn, causes the connected fibrin blood clot to retract and condense as well. Consequently, clot retraction decreases the size of the cut or gash by drawing the damaged ends of the blood vessel toward one another. Fibroblasts and epithelial cells proliferate in and around the clot. This serves to help repair the damaged vessel.

Within several days of clot formation, an enzyme known as **plasmin** completely degrades fibrin, thus dissolving the clot through a process known as **fibrinolysis**. Plasmin is the active form of an inactive plasma protein known as plasminogen. Plasminogen is synthesized and released by the liver and is converted to plasmin by a number of different molecules, one of which is called tissue plasminogen activator (tPA). Endothelial cells produce tPA. In the presence of fibrin, tPA greatly accelerates its enzymatic function to convert plasminogen to plasmin, thus initiating the process of clot dissolution. It is interesting to note that by activating tPA, fibrin initiates its own degradation. tPA can be given to patients who are experiencing heart attacks or strokes caused by an embolism (floating clot). However, the treatment seems to be most effective if it is administered within 90 minutes of onset of symptoms.



Plasmin Activation

Blood Clot Regulation

If the mechanisms governing platelet plug formation and coagulation were allowed to proceed unchecked, undesirable clots would form ultimately resulting in death. The reason that undesirable clots would spontaneously arise is because small amounts of thrombin are always being formed accidentally. Also, there are constant rough areas and small breaks on blood vessels. If clots formed in an unchecked manner, then a clotting cascade could be initiated that would end up in positive feedback and the clotting would grow and develop through the entire vascular system of a person. Just as there are built-in forces the prevent platelet activation that we discussed early in this section, there are also forces that help prevent blood clot formation. Blood plasma contains naturally occurring molecules known as **anticoagulants** which restrict clot formation to locations of damaged vessels.

A plasma protein called **antithrombin** works in conjunction with **heparin** to deactivate thrombin. Heparin is produced on the surface of endothelial cells and released from granules in mast cells. Another anticoagulant produced by endothelial cells is a lipid known as **Prostacyclin** which opposes local concentration of clotting factors by acting as a vasodilator and also targets platelet plug formation by inhibiting platelet activation.

Vitamin K plays an important role in coagulation, as the production of various clotting factors within the liver depends upon this cofactor. A common prescription anticoagulant known as Warfarin (Coumadin) manipulates blood clotting efficiency by inhibiting the activity of an enzyme that participates in recycling vitamin K. The consumption of leafy green vegetables containing high levels of vitamin K interferes with the expected outcomes of this drug.

There are two blood tests used clinically to assess the coagulation of blood, the partial thromboplastin time (PTT) and prothrombin time (PT). The tests are used extensively to help monitor the status of blood clotting in patients being treated with “blood thinners” like heparin inhibition of several intrinsic pathway factors or warfarin (blocks vitamin K actions). The PTT test is used to determine the speed at which blood clots by measuring the effectiveness of clotting factors: VIII, X, XI, and XII. Thus, the PTT test determines if the intrinsic and common pathways are working correctly (heparin treatment). In contrast, the PT test determines the speed of clotting by measuring the effectiveness of factor VII (dependent upon vitamin K) found in the extrinsic pathway (warfarin treatment).