3.1.2

The Stages of Hemostasis

Hemostasis means “to stop the flow of blood” and refers to stopping the movement of blood out of a blood vessel through a lesion. Whenever the integrity of a blood vessel is compromised, blood will leak out and the process of hemostasis is necessary to restore homeostasis within the circulatory system and surrounding tissue. There are four major stages of hemostasis that are discussed in detail below. They are the vessel spasm, platelet plug, clotting cascade, and clot retraction/dissolution.

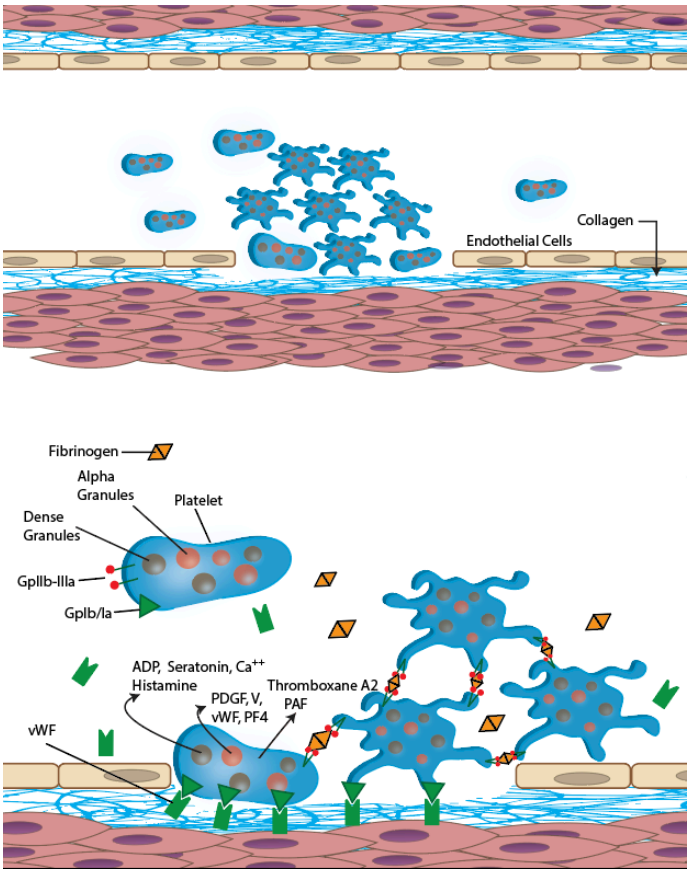
1. Vessel Spasm

The first stage of hemostasis is called the neurogenic mechanism or vessel spasm. This step is characterized by an immediate reflex initiated by nociceptors in the vascular wall. This reflex results in the sympathetic nervous system causing the smooth muscle around the damaged vessel to contract. Damaged endothelial cells also contribute to this contraction by releasing endothelins, which are peptides that cause constriction of vessels by stimulating contraction of the smooth muscle around them.

2. Platelet Plug

Watch the video Platelet Plug Formation – Mechanisms to review platelet plug physiology.

The second stage of hemostasis is the formation of the platelet plug. Platelets, also known as thrombocytes, lack a nucleus and are derived from megakaryocytes. Although they lack a nucleus, they do have a plasma membrane, actin, myosin, mitochondria, and alpha and dense granules. They have an average lifespan of about 8-9 days. Platelet production is increased by the hormone thrombopoietin (TPO). Thrombopoietin is produced by the parenchymal cells and sinusoidal endothelial cells of the liver, the proximal convoluted tubule cells of the kidney, smooth muscle cells, and the stromal cells of the bone marrow. The normal range for platelets in the blood is 150,000-400,000 platelets/microliter. Any value below 150,000 is considered thrombocytopenia.



Platelet Plug Formation *Image by JS W20*

When a vessel experiences damage, smooth muscle and collagen are exposed. The exposure of collagen to the blood allows **von Willebrand factor (vWF)** to bind to it. vWF is produced by healthy endothelial cells and its production is upregulated in damaged endothelium. vWF also binds to GP1B receptors on platelets, effectively forming a link between the platelets and the collagen. This binding causes platelet activation where platelets undergo a conformational change to increase surface area. This change results in a spiky appearance. Activated platelets also release stored vesicles called alpha granules and dense granules. The dense granules contain ADP and calcium which are used to activate nearby platelets. ADP binds to P2Y1 and P2Y12 receptors on platelets which (as previously mentioned) leads to an increase of intracellular calcium. Alpha granules contain additional vWF and platelet derived growth factor (PDGF) which aids in long term wound healing.

TXA2 or **thromboxane** is also released from activated platelets and works to activate other platelets passing by. TXA2 is *not* released from any granules. You might recall that this molecule is a prostaglandin derivative of arachidonic acid that is a product of PLA2 cleaving a phospholipid from the cell membrane. TXA2 binds to TP receptors on platelets, which are G-proteins associated with an intracellular mechanism that uses IP3 to release intracellular stores of calcium. This increased calcium participates in platelet degranulation.

The binding of vWF, ADP, and thromboxane to their respective receptors on platelets causes activation, degranulation, and a conformational shape change of the platelets. Activated platelets also express new proteins on their cell membranes called **GPIIb/GPIIIa** (fibrinogen receptors). Fibrinogen, one of the most plentiful plasma proteins produced by the liver, will bind to two activated platelets via their fibrinogen receptors and link them together. This creates a bridge of fibrinogen anchored to two separate platelets via the GPIIb/GPIIIa receptors. This interaction makes platelets stick to one another in a process called platelet aggregation and forms a platelet plug which can seal small breaks in vessel walls. This platelet plug will also act as a scaffolding structure for fibrin strands to adhere and wrap around during the clotting cascade stage of hemostasis.

An additional molecule you should be aware of is **platelet activating factor (PAF)**. PAF is a type of phospholipid that is generated by platelets, endothelial cells, and several immune cells. The binding of PAF to a G-protein on platelets results in platelet activation. PAF is produced in greater quantity during times of inflammation. Because of its increase during inflammation, this molecule helps us understand one of the ways that inflammation can increase the risk of platelet plug and clot formation.

Another way that platelets can be activated is via thrombin, which is created when prothrombin is activated in the clotting cascade. Thrombin binds to the PAR-1 receptor on platelets and leads to their activation.

Several medications can interfere with the formation of platelet plugs. NSAIDS (non-steroidal anti-inflammatory drugs) will block the enzyme inside of platelets called cyclooxygenase-1 (COX-1). This in turn will decrease production of prostaglandins including TXA2. Clopidogrel (Plavix) and Ticlopidine (Ticlid) are drugs that block the P2Y1 and P2Y12 (ADP receptors) on platelets. Abciximab (Reopro), Eptifibatide (Integrilin), and Tirofiban (Aggrastat) block the GPIIb/IIIa fibrinogen receptor on platelets. Vorapaxar (Zontivity) is a PAR-1 (thrombin receptor) blocker.

3. Clotting/Coagulation Cascade

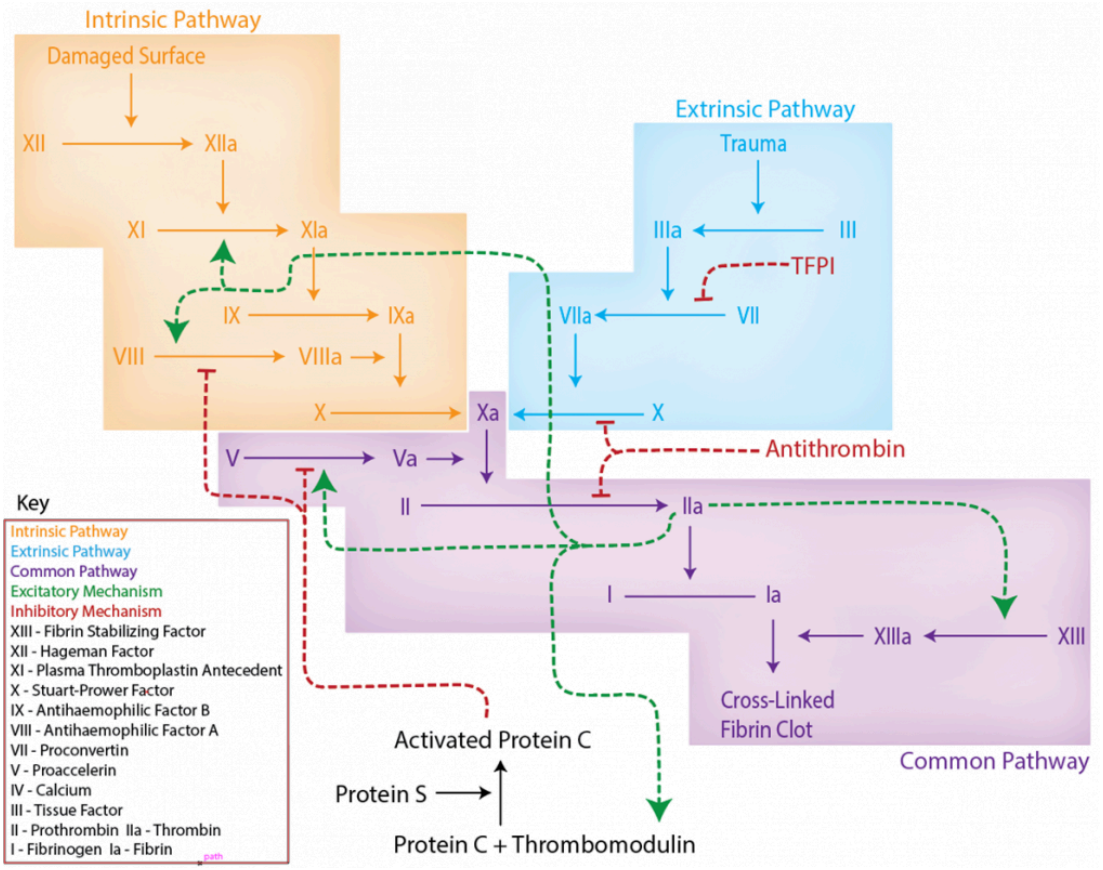


Image by Becky T. S20

The third stage of hemostasis is the activation of the clotting cascade and clotting factors. The clotting cascade can begin with either of two pathways: intrinsic and extrinsic. These two pathways eventually merge into the common pathway through the activation of factor X.

The intrinsic pathway begins when collagen becomes exposed in the vessel wall. Collagen has a negative charge which facilitates the activation of factor XII. Factor XIIa activates factor XI, which in turn activates factor IX and factor IXa recruits cofactor VIII. The factor IXa/cofactor VIII complex combines with calcium and negatively charged phospholipids on a platelet to form a larger complex that is capable of activating factor X, thereby initiating the common pathway.

The extrinsic pathway begins when tissues experience damage/trauma. With trauma, capillaries and small blood vessels may rupture and expose blood to a protein found outside of the blood vessels called tissue factor or TF (also called tissue thromboplastin or factor III). Tissue factor is expressed by many cells surrounding blood vessels including macrophages, dendritic cells, and smooth muscle cells. As blood passes through these damaged tissues, circulating molecules of factor VII associate with a combination of TF, phospholipids and calcium to form an enzymatic complex. This complex reacts with and activates factor X and the common pathway is again initiated. Note: the factor III/ VII phospholipid/calcium complex is also capable of activating factor IX within the intrinsic pathway.

As mentioned, the common pathway starts with the activation of factor X into Xa. Factor Xa then recruits a cofactor called factor V to form the factor X/factor V complex that is able to transform inactive prothrombin (factor II) to active thrombin (factor IIa). Thrombin in turn activates fibrinogen (factor I) to fibrin (Ia). Fibrin is then turned into a cross-linked fibrin clot with the help of factor XIII.

Anticoagulant Drugs and Tests Used to Monitor Them:

The clotting cascade is affected by several anticoagulant drugs. Our discussion will center around two of the most common: heparin and warfarin. These anticoagulant drugs are used in the treatment and prevention of venous thrombotic events (VTE) such as deep vein thrombosis (DVT) and pulmonary embolism (PE).

Heparin increases the activity of antithrombin III (AT-III). Synthetic heparin and heparin-like molecules must be given intravenously because they cannot be absorbed from the GI tract. Heparin can be given as a larger polysaccharide molecule called **unfractionated heparin (UFH)** or a smaller, short chain polysaccharide called **low molecular weight heparin (LMWH)** or fractionated heparin. The differences between these two forms of heparin include:

- UFH is often given by IV infusion rather than by subcutaneous injection.
- LMWH can be given at home because it does not have to be monitored as closely whereas UFH is generally given to patients staying in the hospital so its effects can be closely monitored.
- LMWH has a smaller risk of osteoporosis as a side effect with long-term use.
- There is a smaller risk of HIT and HITT with LMWH.
- LMWH is cleared by the kidney and so is sometimes contraindicated for patients with kidney disease.

Warfarin (or Coumadin) is an oral drug that inhibits the production of several clotting factors. Many clotting factors are vitamin K dependent, which means that they need vitamin K in order to be produced in the liver. An enzyme called vitamin K reductase turns vitamin K into its reduced form which is then used in the production of several clotting factors. Warfarin inhibits vitamin K reductase so that the reduced form of vitamin K is not made and thus clotting factors cannot be synthesized. Reduced vitamin K is necessary to form factors II, VII, IX, and X, as well as protein C and S (explained earlier). This drug works slower than heparin because it prevents the formation of clotting factors rather than just decreasing the activity of preexisting clotting factors. Warfarin takes 36-72 hours for maximum effect because of the variability in the half-lives of the coagulation factors that remain in circulation.

There are different tests performed on patients undergoing anticoagulant therapy to monitor therapy effectiveness and keep clotting time within a desirable range. We will discuss them now.

- **Prothrombin time (PT time)** is used to monitor warfarin therapy and assess the extrinsic and common pathways of the coagulation cascade. For this test, plasma from a patient's blood sample is obtained and citrate is added. Citrate binds up calcium so that the clotting cascade is not successful in making fibrin before the lab test begins. Excess calcium and thromboplastin (TF) are then added to the citrated plasma and the time it takes to clot is measured. While PT is measured in seconds, the results are given in the form of an **INR** (international normalized ratio). This is because different labs have slightly different protocols and so the absolute number of seconds to clot can differ. Therefore the lab establishes a normal number of seconds and then divides that into the test they perform. For example, if a sample of blood took 22 seconds to clot but the normal number of seconds for that lab was 11 seconds, then a ratio is measured where 11 is divided into 22 and the INR would be 2. The normal PT of an individual not on anticoagulant therapy is generally 11 to 13.5 seconds, which corresponds to an INR of 0.8 to 1.1. For a patient who is prone to clots and is taking an anticoagulant drug, the target INR is 2-3.
- **Activated partial thromboplastin time (aPTT)** is used to monitor heparin (UFH) therapy and assess the *intrinsic* and common pathways. For this test, plasma is obtained from a patient's blood sample and citrate is added to bind up all the calcium so clotting factors do not become activated before the lab test begins. Excess calcium and kaolin and phospholipids (both of which have a negative charge that mimics the negative charge of collagen that begins the intrinsic pathway) are added to the citrated plasma and then the time it takes to clot is measured. For someone who is not taking anticoagulant medication, the normal range for aPTT is 30-40 seconds. For a patient on anticoagulant therapy, the goal is to achieve an aPTT of about 1.5 to 2.5 times the normal value.
- **Anti-factor Xa activity test** is used to monitor LMWH therapy because it behaves differently than UFH. Interestingly, while UFH is known to activate antithrombin's effects against several of the activated intrinsic clotting factors, LMWH only inhibits factor X. LMWH is a shorter molecule and this simple difference is enough to make it so that LMWH does not promote molecular binding of antithrombin with most of the intrinsic clotting factors. However, the length of LMWH does successfully promote good binding of antithrombin with factor X. Therefore, if a patient is on LMWH therapy, the aPTT is not a good test to use because it monitors the intrinsic pathway. Instead, individuals on LMWH therapy are preferably assessed using a test called the anti-factor Xa activity test. In this test no clotting time is assessed. Instead, a patient's plasma is added to a known amount of antithrombin to create a functional heparin-antithrombin complex that binds to factor Xa. A chromogenic substrate that causes color if it binds Xa is also added to the test. If there is a lot of LMWH in the patient's blood, much of the factor Xa will be bound and less free Xa will be available for the chromogenic substrate and the color will be lighter. If there is less LMWH in the patient's blood, then there will be more of the added Xa available for the chromogenic substrate and the color will be darker. This visual test helps determine if more or less LMWH should be used to maintain a desired anticoagulant effect in LMWH therapy.

4. Clot Retraction/Dissolution

The fourth and final stage of hemostasis is clot retraction/dissolution. This stage is when the body removes formed clots that are no longer necessary because healthy endothelial cells are covering the lesion and the clotting processes have ceased. An important protein in clot dissolution is plasminogen, which is synthesized and released by the liver. **Plasminogen** is activated into **plasmin** by tPA and goes on to break down fibrin. Interestingly, tPA uses fibrin as a cofactor in this reaction. Because of this, the increased production of fibrin sets the stage for its own destruction because it leads to an increase of the production of plasmin. An enzyme called **urokinase** is also very capable of reacting with plasminogen to make plasmin. Urokinase is made in very small amounts by the endothelial cells since these cells make much more tPA. However, cells in the kidney make a lot more urokinase and it is likely much more important there to dissolve fibrin strands.

Synthetic forms of tPA and urokinase have been developed and are used to help dissolve blood clots. While nicknamed the "clot busters," a more formal description for these drugs is thrombolytic or fibrinolytic therapy. If an unwanted clot has occurred in the body, thrombolytic therapy should be used quickly to restore blood flow because waiting too long can result in tissue ischemia and irreversible damage. However, thrombolytic therapy is very dangerous to use in individuals who are taking drugs that prevent clotting or are suspected to have any pathology that decreases clotting times because of increased bleeding risk.

Now we are going to discuss various blood disorders in detail. As a general overview, this information is organized according to whether the conditions involve platelets are not. They are also grouped according to if the condition is inherited genetically (primary) or acquired throughout life (secondary).