Platelets are not cells in the strict sense. About one-fourth the diameter of a lymphocyte, they are cytoplasmic fragments of extraordinarily large cells (up to 60 µm in diameter) called megakaryocytes. In blood smears, each platelet exhibits a blue-staining outer region and an inner area containing granules that stain purple. The granules contain an impressive array of chemicals that act in the clotting process, including serotonin, Ca\(^{2+}\), a variety of enzymes, ADP, and platelet-derived growth factor (PDGF) and thromboxane A\(_2\).

Platelets are essential for the clotting process that occurs in plasma when blood vessels are ruptured or their lining is injured. By sticking to the damaged site, platelets form a temporary plug that helps seal the break. Because platelets are anucleate, they age quickly and degenerate in about ten days if they are not involved in clotting. In the meantime, they circulate freely, kept mobile but inactive by molecules (nitric oxide, prostacyclin) secreted by endothelial cells lining the blood vessels.

Platelet formation is regulated by a hormone called thrombopoietin. Their immediate ancestral cells, the megakaryocytes, are progeny of the hemocytoblast and the myeloid stem cell, but their formation is quite unusual.

In this line, repeated mitoses of the megakaryoblast occur, but cytokinesis does not. The final result is the megakaryocyte, a bizarre cell with a huge, multilobed nucleus and a large cytoplasmic mass. When formed, the megakaryocyte presses up against a sinusoid (the specialized type of capillary in the marrow) and sends cytoplasmic extensions through the sinusoid wall into the bloodstream. These extensions rupture, releasing the platelet fragments like stamps being torn from a sheet of postage stamps and seeding the blood with platelets. The plasma membranes associated with each fragment quickly seal around the cytoplasm to form the grainy, roughly disc-shaped platelets, each with a diameter of 2–4 µm. Each cubic millimeter of blood contains between 150,000 and 400,000 of the tiny platelets.

**Hemostasis**

Normally, blood flows smoothly past the intact blood vessel lining (endothelium). But if a blood vessel wall breaks, a whole series of reactions is set in motion to accomplish hemostasis, or stoppage of bleeding. Without this plug-the-hole defensive reaction, we would quickly bleed out our entire blood volume from even the smallest cuts.

The hemostasis response, which is fast, localized, and carefully controlled, involves many blood coagulation factors normally present in plasma as well as some substances that are released by platelets and injured tissue cells.
During hemostasis, three steps occur in rapid sequence:
(1) vascular spasms
(2) platelet plug formation, and
(3) coagulation, or blood clotting

Blood loss at the site is permanently prevented when fibrous tissue grows into the clot and seals the hole in the blood vessel.

**Vascular Spasms**

The immediate response to blood vessel injury is constriction of the damaged blood vessel (vasoconstriction). Factors that trigger this vascular spasm include direct injury to vascular smooth muscle, chemicals released by endothelial cells and platelets, and reflexes initiated by local pain receptors. The spasm mechanism becomes more and more efficient as the amount of tissue damage increases, and is most effective in the smaller blood vessels. The value of the spasm response is obvious: A strongly constricted artery can significantly reduce blood loss for 20–30 minutes, allowing time for platelet plug formation and blood clotting to occur.
In the normal environment the outside of the platelets are positively charged. The lining of the endothelium inside the blood vessel also contains a chemical called prostacyclin which creates a positive charge of the membrane. The end result is that the platelets and the lining of the blood vessel all repel one another. However, when an injury occurs and the vessel and surrounding tissue are cut there is a release of collagen, a negatively charged protein that then attracts the platelet. With an initial cut of the vessel, particularly in cross section, the vessel’s smooth muscle responds by constricting – vascular spasm – thus reducing the amount of blood.

**Platelet Release Reaction**

Following the vascular spasm a subsequent series of reactions occur called the **platelet release reactions**. With the injury the platelets drawn to the injury site by the change in charge are exposed to $N_2$ and fracture releasing three groups of substances.

**Serotonin + thromboxane $A_2$** - these substances are vasoconstrictors and enhance the constriction of the vessel at the site of the injury thus reducing the overall blood loss.

**ADP** – its release triggers a greater attraction to platelets to the site of injury which subsequently fracture and release more thromboxane and serotonin and ADP attracting more platelets etc.etc. The result is the formation of a water soluble **white platelet plug**. This you have seen – it is the somewhat mucoid, gelatinous material that initially forms over an injury such as a scrape. If washed, it dissolves and the mechanism begins again from scratch.

**Phospholipids** - this release from platelets sets up, activates and establishes the formal clotting mechanism.

**CLOTTING MECHANISM**

Coagulation or blood clotting during which blood is transformed from a liquid to a gel, is a multistep process that leads to its critically important last three phases:

- A complex substance called prothrombin activator is formed.
- Prothrombin activator converts a plasma protein called prothrombin into thrombin, an enzyme.
- Thrombin catalyzes the joining of fibrinogen molecules present in plasma to a fibrin mesh, which traps blood cells and effectively seals the hole until the blood vessel can be permanently repaired.

The complete coagulation process is much more complicated, however. Over 30 different substances are involved. Factors that enhance clot formation are called clotting factors or procoagulants. Although vitamin K is not directly involved in coagulation, this fat-soluble vitamin is required for the synthesis of four of the procoagulants made by the liver. Factors that inhibit clotting are called anticoagulants. Whether or not blood clots depends on a delicate balance between these two groups of factors. Normally, anticoagulants dominate and clotting is prevented; but when a vessel is ruptured, procoagulant activity in that area increases dramatically and clot formation begins. The procoagulants are numbered I to XIII according to the order of their discovery; hence the numerical order does not reflect the reaction sequence. Tissue factor (III) and Ca$^2+$ (IV) are usually indicated by their names, rather than by numerals. Most of these factors are plasma proteins made by the liver that circulate in an inactive form in blood until mobilized.

**Phase 1: Two Pathways to Prothrombin Activator**

Clotting may be initiated by either the intrinsic or the extrinsic pathway, and in the body both pathways are usually triggered by the same tissue-damaging events. Clotting of blood outside the body is initiated only by the intrinsic mechanism.
Pivotal components in both mechanisms are negatively charged membranes, particularly those on platelets containing phosphatidylserine, also known as PF3 (platelet factor 3). Many intermediates of both pathways can be activated only in the presence of PF3. In the slower intrinsic pathway, all factors needed for clotting are present in (intrinsic to) the blood. By contrast, when blood is exposed to an additional factor in tissues underneath the damaged endothelium called tissue factor (TF), factor III, or tissue thromboplastin, the “shortcut” extrinsic mechanism, which bypasses several steps of the intrinsic pathway, is triggered.

Each pathway requires calcium and involves the activation of a series of procoagulants, each functioning as an enzyme to activate the next procoagulant in the sequence. The intermediate steps of each pathway cascade toward a common intermediate, factor X. Once factor X has been activated, it complexes with calcium ions, PF3, and factor V to form prothrombin activator. This is usually the slowest step of the blood clotting process, but once prothrombin activator is present, the clot forms in 10 to 15 seconds.

Phase 2: Common Pathway to Thrombin
Prothrombin activator catalyzes the transformation of the plasma protein prothrombin to the active enzyme thrombin.

Phase 3: Common Pathway to the Fibrin Mesh
Thrombin catalyzes the polymerization of fibrinogen (another plasma protein made by the liver). As the fibrinogen molecules are aligned into long, hairlike, insoluble fibrin strands, they glue the platelets together and make a web that forms the structural basis of the clot. In the presence of fibrin, plasma becomes gel-like and traps formed elements that try to pass through it.

In the presence of calcium ions, thrombin also activates factor XIII (fibrin stabilizing factor), a cross-linking enzyme that binds the fibrin strands tightly together and strengthens and stabilizes the clot. Clot formation is normally complete within 3 to 6 minutes after blood vessel damage. Because the extrinsic pathway involves fewer steps it is more rapid than the intrinsic pathway; in cases of severe tissue trauma it can promote clot formation within 15 seconds.
Intrinsic pathway:
Vessel endothelium ruptures, exposing underlying tissues (e.g., collagen)

Platelets cling and their surfaces provide sites for mobilization of factors

\[ \text{XII} \rightarrow \text{XII}_a \rightarrow \text{Xa} \rightarrow \text{IX}_a \rightarrow \text{IXa/VIII}_a \text{ complex} \]

Extrinsic pathway:
Tissue cell trauma causes exposure of blood to

\[ \text{Tissue factor (TF)} \]

\[ \text{Ca}^{2+} \rightarrow \text{VII} \rightarrow \text{VII}_a \rightarrow \text{VIII} \rightarrow \text{VIII}_a \rightarrow \text{IXa/VIII}_a \text{ complex} \]

\[ \text{PF}_3 \text{ released by aggregated platelets} \]

\[ \text{IXa/VIII}_a \text{ complex} \rightarrow \text{Xa} \rightarrow \text{X} \rightarrow \text{V} \rightarrow \text{Va} \rightarrow \text{Prothrombin activator} \]

\[ \text{Prothrombin (II)} \rightarrow \text{Thrombin (IIa)} \rightarrow \text{Fibrinogen (I)} \rightarrow \text{Fibrin} \rightarrow \text{XIII} \rightarrow \text{XIII}_a \rightarrow \text{Cross-linked fibrin polymer} \]
Clot Retraction and Repair - Syneresis

Within 30 to 60 minutes, the clot is stabilized further by a platelet-induced process called clot retraction. Platelets contain contractile proteins (actin and myosin), and they contract in much the same manner as muscle cells. As the platelets contract, they pull on the surrounding fibrin strands, squeezing serum (plasma minus the clotting proteins) from the mass, compacting the clot and drawing the ruptured edges of the blood vessel more closely together. Even as clot retraction is occurring, vessel healing is taking place. Platelet-derived growth factor (PDGF) released by platelet degranulation stimulates smooth muscle cells and fibroblasts to divide and rebuild the wall. As fibroblasts form a connective tissue patch in the injured area, endothelial cells, stimulated by vascular endothelial growth factor (VEGF), multiply and restore the endothelial lining.

Fibrinolysis

A clot is not a permanent solution to blood vessel injury, and a process called fibrinolysis removes unneeded clots when healing has occurred. Because small clots are formed continually in vessels throughout the body, this cleanup detail is crucial. Without fibrinolysis, blood vessels would gradually become completely blocked.

The critical natural “clot buster” is a fibrin-digesting enzyme called plasmin, which is produced when the plasma protein plasminogen is activated. Large amounts of plasminogen are incorporated into a forming clot, where it remains inactive until appropriate signals reach it. The presence of a clot in and around the blood vessel causes the endothelial cells to secrete tissue plasminogen activator (tPA). Activated factor XII and thrombin released during clotting also serve as plasminogen activators. As a result, most plasmin activity is confined to the clot, and any plasmin that strays into the plasma is quickly destroyed by circulating enzymes. Fibrinolysis begins within two days and continues slowly over several days until the clot is finally dissolved.

Factors Limiting Normal Clot Growth

Once the clotting cascade has begun, it continues until a clot is formed. Normally, two homeostatic mechanisms prevent clots from becoming unnecessarily large: (1) swift removal of clotting factors, and (2) inhibition of activated clotting factors. For clotting to occur in the first place, the concentration of activated procoagulants must reach certain critical levels. Clot formation in rapidly moving blood is usually curbed because the activated clotting factors are diluted and washed away. For the same reasons, further growth of a forming clot is hindered when it contacts blood flowing normally.

Other mechanisms block the final step in which fibrinogen is polymerized into fibrin by restricting thrombin to the clot or by inactivating it if it escapes into the general circulation. As a clot forms, almost all of the thrombin produced is bound onto the fibrin threads. This is an important safeguard because thrombin also exerts positive feedback effects on the coagulation process prior to the common pathway. Not only does it speed up the production of prothrombin activator by acting indirectly through factor V, but it also accelerates the earliest steps of the intrinsic pathway by activating platelets. Thus, fibrin effectively acts as an anticoagulant to prevent enlargement of the clot and prevents thrombin from acting elsewhere. Thrombin not bound to fibrin is quickly inactivated by antithrombin III, a protein present in plasma. Antithrombin III and protein C, another protein produced in the liver, also inhibit the activity of other intrinsic pathway procoagulants.

Heparin, the natural anticoagulant contained in basophil and mast cell granules, is also found on the surface of endothelial cells. It inhibits thrombin by enhancing the activity of antithrombin III. Like most other clotting inhibitors, heparin also inhibits the intrinsic pathway.