Hemostasis
Hemostasis and Thrombosis

*Normal hemostasis* is a consequence of tightly regulated processes that maintain blood in a fluid state in normal vessels, yet also permit the rapid formation of a *hemostatic clot* at the site of a vascular injury.

*Thrombosis* involves blood clot formation within intact vessels. Both hemostasis and thrombosis involve three components: the vascular wall, platelets and the coagulation cascade.
Elements of the Hemostatic process

- Endothelium
- Anti-thrombosis
- Pro-thrombosis
- Platelets
- Platelet-endothelial cell interaction
- Coagulation cascade

FIGURE 4–8 The coagulation cascade. Factor IX can be activated either by factor Xlla or factor VIIa; in lab tests, activation is predominantly dependent on factor Xlla of the intrinsic pathway. Factors in red boxes represent inactive molecules; activated factors are indicated with a lower case “a” and a green box. Note also the multiple points where thrombin (factor IIa; light blue boxes) contributes to coagulation through positive feedback loops. The red “X”s denote points of action of tissue factor pathway inhibitor (TFPI), which inhibits the activation of factors X and IX by factor VIIa. PL, phospholipid; HMWK, high-molecular-weight kininogen.

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After initial injury there is a brief period of arteriolar vasoconstriction mediated by reflex neurogenic mechanisms and augmented by the local secretion of factors such as endothelin (a potent endothelium-derived vasoconstrictor).

The effect is transient, however, and bleeding would resume if not for activation of the platelet and coagulation systems.
Endothelial injury exposes highly thrombogenic subendothelial extracellular matrix (ECM), facilitating platelet adherence and activation. Activation of platelets results in a dramatic shape change (from small rounded discs to flat plates with markedly increased surface area), as well as the release of secretory granules. Within minutes the secreted products recruit additional platelets (aggregation) to form a hemostatic plug; this process is referred to as primary hemostasis.
Tissue factor is also exposed at the site of injury. Also known as factor III and thromboplastin, tissue factor is a membrane-bound procoagulant glycoprotein synthesized by endothelial cells. It acts in conjunction with factor VII (see below) as the major in vivo initiator of the coagulation cascade, eventually culminating in thrombin generation. Thrombin cleaves circulating fibrinogen into insoluble fibrin, creating a fibrin meshwork, and also induces additional platelet recruitment and activation. This sequence, secondary hemostasis, consolidates the initial platelet plug.
Polymerized fibrin and platelet aggregates form a solid, permanent plug to prevent any further hemorrhage. At this stage, counter-regulatory mechanisms (e.g., tissue plasminogen activator, t-PA) are set into motion to limit the hemostatic plug to the site of injury.
Endothelial cells are key players in the regulation of homeostasis, as the balance between the anti- and prothrombotic activities of endothelium determines whether thrombus formation, propagation, or dissolution occurs.

Normally, endothelial cells exhibit antiplatelet, anticoagulant, and fibrinolytic properties.

After injury or activation they acquire numerous procoagulant activities.
FIGURE 4–6 Anti- and procoagulant activities of endothelium. NO, nitric oxide; PGI₂, prostacyclin; t-PA, tissue plasminogen activator; vWF, von Willebrand factor. The thrombin receptor is also called a protease-activated receptor (PAR).

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Intact endothelium prevents platelets (and plasma coagulation factors) from engaging the highly thrombogenic subendothelial ECM.

Non-activated platelets do not adhere to endothelial cells, and even if platelets are activated, prostacyclin (PGI2) and nitric oxide produced by the endothelial cells impede platelet adhesion.

PGI2 and NO are potent vasodilators and inhibitors of platelet aggregation; their synthesis by the endothelium is stimulated by several factors produced during coagulation (e.g., thrombin and cytokines). Endothelial cells also elaborate adenosine diphosphatase, which degrades adenosine diphosphate (ADP) and further inhibits platelet aggregation (see below).
Anticoagulant effects

Mediated by endothelial membrane-associated heparin-like molecules, thrombomodulin, and tissue factor pathway inhibitor.

The heparin-like molecules act indirectly; they are cofactors that greatly enhance the inactivation of thrombin and several other coagulation factors by the plasma protein antithrombin III.

Thrombomodulin binds to thrombin and converts it from a procoagulant into an anticoagulant via its ability to activate protein C, which inhibits clotting by inactivating factors Va and VIIIa.

Endothelium also produces protein S, a co-factor for protein C, and tissue factor pathway inhibitor (TFPI), a cell surface protein that directly inhibits tissue factor–factor VIIa and factor Xa activities.
Thrombin and Fibrinolysis*

The balance between the formation and degradation of FN. The coagulation cascade ultimately generates thrombin, which catalyzes the conversion of fibrinogen to the fibrin clot. The fibrinolytic cascade generates plasmin, which catalyzes solubilization of the FN. The thrombin-thrombomodulin complex promotes down-regulation of thrombin formation by generating activated protein C (APC). It also suppresses fibrinolysis by forming TAFIa. The two cascades are thereby linked through the thrombin, thrombomodulin, and TAFI pathway. PC = protein C.

Figure Legend:

The balance between the formation and degradation of FN. The coagulation cascade ultimately generates thrombin, which catalyzes the conversion of fibrinogen to the fibrin clot. The fibrinolytic cascade generates plasmin, which catalyzes solubilization of the FN. The thrombin-thrombomodulin complex promotes down-regulation of thrombin formation by generating activated protein C (APC). It also suppresses fibrinolysis by forming TAFIa. The two cascades are thereby linked through the thrombin, thrombomodulin, and TAFI pathway. PC = protein C.
Endothelial cells synthesize tissue-type plasminogen activator (t-PA), a protease that cleaves plasminogen to form plasmin; plasmin, in turn, cleaves fibrin to degrade thrombi.
Prothrombotic Properties

While normal endothelial cells limit clotting, trauma and inflammation of endothelial cells induce a prothrombotic state that alters the activities of platelets, coagulation proteins, and the fibrinolytic system.
Platelet Effects

Endothelial injury allows platelets to contact the underlying extracellular matrix; subsequent adhesion occurs through interactions with von Willebrand factor (vWF), which is a product of normal endothelial cells and an essential cofactor for platelet binding to matrix elements.

![Diagram showing platelet effects](source: Am J Health-Syst Pharm © 2002 American Society of Health-System Pharmacists)
Antifibrinolytic Effects

Endothelial cells secrete inhibitors of plasminogen activator (PAIs), which limit fibrinolysis and tend to favor thrombosis.
Platelets

Platelets are disc-shaped, anucleate cell fragments that are shed from megakaryocytes in the bone marrow into the bloodstream.

They play a critical role in normal hemostasis by forming the hemostatic plug that initially seals vascular defects, and by providing a surface that recruits and concentrates activated coagulation factors.

Their function depends on several glycoprotein receptors, a contractile cytoskeleton, and two types of cytoplasmic granules:

- α-Granules have the adhesion molecule P-selectin on their membranes and contain fibrinogen, fibronectin, factors V and VIII, platelet factor 4 (a heparin-binding chemokine), platelet-derived growth factor (PDGF), and transforming growth factor-β (TGF-β).

- Dense (or δ) granules contain ADP and ATP, ionized calcium, histamine, serotonin, and epinephrine.
Release of the contents of dense-bodies is especially important, since calcium is required in the coagulation cascade, and ADP is a potent activator of platelet aggregation. ADP also begets additional ADP release, amplifying the aggregation process.
Megakaryocyte in Bone Marrow

http://path.upmc.edu/cases/case37/images/micro8.jpg

http://www.med-ed.virginia.edu/courses/path/innes/images/nhjpeg/nh%20megakaryocyte%20x50a.jpeg

http://www.hematology.org/assets/0/71/73/78/135/137/238/8c1482dc-1d4c-4ea5-b199-aca3f01eac83.jpg?n=8006

http://upload.wikimedia.org/wikipedia/commons/7/7f/Platelets_by_budding_off_from_megakaryocytes.jpg

Hematopoietic stem cell

Promegakaryocyte

Megakaryocyte

Platelets

Blood vessel in bone marrow

Shear flow

Sinusoidal endothelial cell

Developing blood cell

Megakaryocyte

Proplatelets
Platelet structure
Platelet Actions

- Adhesion to extracellular matrix
  - GpIb links to collagen via vWF
  - GpIIb/IIIa links platelets via fibrinogen
- Secretion from granules into canaliculi and exterior
- Transition of phospholipids to outer lamina
- Aggregation
  - Primary hemostatic plug
- Contraction
  - Secondary hemostatic plug
Platelet aggregation
After vascular injury, platelets encounter ECM constituents such as collagen and the adhesive glycoprotein vWF. On contact with these proteins, platelets undergo:
(1) adhesion and shape change,
(2) secretion (release reaction)
(3) aggregation

Platelet adhesion to ECM is mediated largely via interactions with vWF, which acts as a bridge between platelet surface receptors (Glycoprotein Ib-IX-V complex (GPIb-IX-V)) and exposed collagen.

Although platelets can also adhere to other components of the ECM (e.g., fibronectin), vWF-GPIb associations are necessary to overcome the high shear forces of flowing blood.
Platelet aggregation follows adhesion and granule release.

In addition to ADP, the vasoconstrictor thromboxane A2 is an important platelet-derived stimulus that amplifies platelet aggregation, which leads to the formation of the primary hemostatic plug.

Although this initial wave of aggregation is reversible, concurrent activation of the coagulation cascade generates thrombin, which stabilizes the platelet plug via two mechanisms.

First, thrombin binds to a protease-activated receptor (PAR, see below) on the platelet membrane and in concert with ADP and TxA2 causes further platelet aggregation.

This is followed by platelet contraction, an event that is dependent on the platelet cytoskeleton that creates an irreversibly fused mass of platelets, which constitutes the definitive secondary hemostatic plug. Second, thrombin converts fibrinogen to fibrin in the vicinity of the platelet plug, functionally cementing the platelets in place.
FIGURE 4–7 Platelet adhesion and aggregation. Von Willebrand factor functions as an adhesion bridge between subendothelial collagen and the glycoprotein Ib (GpIb) platelet receptor. Aggregation is accomplished by fibrinogen bridging GpIb-IIIa receptors on different platelets. Congenital deficiencies in the various receptors or bridging molecules lead to the diseases indicated in the colored boxes. ADP, adenosine diphosphate.

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Fig. 2  Platelet adhesion to the injured vessel wall. At sites of vascular injury and endothelial denudation, platelets adhere to the subendothelial matrix. Platelet surface receptors GPIa/IIa, GPIV and GPVI interact directly with collagen, GPIb/V...
Fig. 3  Platelet?endothelial cell interaction. Upon activation, endothelial cells express P-selectin which facilitates platelet rolling via GPIbα and possibly via PSGL-1. Following a conformational change, ? IIb ? 3 integrins establish firm adhes...
Platelet-Endothelial Cell Interactions

The **endothelial cell-derived prostaglandin PGI<sub>2</sub>** (prostacyclin) inhibits **platelet aggregation** and is a potent vasodilator; conversely, the **platelet-derived prostaglandin TxA<sub>2** activates platelet aggregation** and is a vasoconstrictor.

Effects mediated by PGI<sub>2</sub> and TxA<sub>2</sub> are balanced to effectively modulate platelet and vascular wall function: at baseline, platelet aggregation is prevented, whereas endothelial injury promotes hemostatic plug formation.
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