Blood clotting mechanism

Blood clotting (hemostasis) is a tightly regulated, multi-step process that stops bleeding while keeping blood fluid elsewhere. It is classically divided into:

- Vascular response
- Primary hemostasis (platelet plug)
- Secondary hemostasis (coagulation cascade → fibrin clot)
- Clot stabilization and fibrinolysis

1. Vascular and endothelial response

When a blood vessel is injured:

- **Vasoconstriction**: Local smooth muscle contracts (neurogenic reflexes + endothelin), reducing blood flow and immediate blood loss.
- **Loss of endothelial antithrombotic properties**:
  - Healthy endothelium normally secretes **NO** and **prostacyclin (PGI_2)**, which inhibit platelet activation and aggregation.
  - It also expresses **heparan sulfate** (enhances antithrombin), **thrombomodulin** (activates protein C), and **tissue plasminogen activator (tPA)**, all of which are anticoagulant/fibrinolytic.
- Injury exposes **subendothelial collagen** and **von Willebrand factor (vWF)**, converting the surface from anti- to pro-thrombotic.

This sets the stage for primary hemostasis.

2. Primary hemostasis: formation of the platelet plug

Primary hemostasis is rapid and mainly platelet-dependent. It proceeds through:

2.1 Platelet adhesion

1. **Endothelial injury** exposes:
   - Subendothelial **collagen**
   - Immobilized **vWF** bound to collagen.
2. Circulating platelets tether and adhere via:
   - Platelet **GPIb-IX-V receptor** binding to vWF → crucial for high shear vessels.
   - Direct collagen receptors (**GPVI, integrin α2β1**) also participate.

Defects:

- Bernard–Soulier syndrome → defective GPIb → impaired adhesion.
- vWD → decreased/abnormal vWF → defective adhesion and also factor VIII carriage.

2.2 Platelet activation

Once adhered, platelets are activated:

- **Shape change**: Discoid platelets become spiky spheres, increasing surface area.
- **Degranulation**:
  - **Dense granules** release ADP, Ca^{2+}, serotonin.
  - **Alpha granules** release fibrinogen, vWF, factor V, platelet-derived growth factor, etc.
- **Synthesis of thromboxane A_2 (TXA_2)** via COX-1:
  - TXA_2 is a potent vasoconstrictor and platelet activator.
- **Receptor upregulation**:
  - Activated platelets express the integrin **GPIIb/IIIa (αIIbβ3)** in high-affinity form, which binds fibrinogen and vWF, essential for aggregation.
- **Exposure of phosphatidylserine (PS)** on the outer membrane:
  - PS provides a **negatively charged surface** for assembly of coagulation factor complexes (tenase, prothrombinase), greatly amplifying secondary hemostasis.

2.3 Platelet aggregation → primary platelet plug

- Released **ADP** and **TXA_2** recruit and activate nearby platelets.
- **Fibrinogen** (and vWF) bridges between GPIIb/IIIa on adjacent platelets, causing aggregation.
- A **primary (temporary) platelet plug** forms, sufficient for very small vessels but mechanically weak and easily dislodged.

This plug then becomes the scaffold on which fibrin is laid down during secondary hemostasis.

3. Secondary hemostasis: coagulation cascade and fibrin formation

Secondary hemostasis stabilizes the platelet plug by depositing a **fibrin mesh**. Classically, the cascade is described in three pathways:

- Extrinsic (tissue factor) pathway
- Intrinsic (contact activation) pathway
- Common pathway

In vivo, they are highly interconnected, but the separation is useful conceptually and for lab tests (PT vs aPTT).

3.1 Key principles

- Most coagulation factors are zymogens that become **serine proteases** when activated.
- Reactions occur on **phospholipid surfaces** (mainly activated platelets) in the presence of **Ca^{2+}**, limiting clotting to the injury site.
- **Vitamin K-dependent factors** (II, VII, IX, X, and proteins C, S, Z) require γ-carboxylation of glutamate residues in the liver; this modification enables Ca^{2+}-dependent binding to phospholipid membranes.

4. Extrinsic pathway (tissue factor pathway)

The extrinsic pathway is the **rapid initiator** of coagulation.

1. **Tissue factor (TF; factor III)**:
    - A transmembrane protein in subendothelial cells, smooth muscle, fibroblasts.
    - Exposed to blood upon vascular injury.
2. In plasma, **factor VII** binds TF and is activated to **FVIIa**.
3. **TF–FVIIa complex** (on a membrane, with Ca^{2+}) activates:
    - **Factor X → Xa** (main route)
    - **Factor IX → IXa** (provides linkage to intrinsic pathway).
4. Generated **FXa** enters the **common pathway** to produce a "small burst" of thrombin.

Clinical: The **prothrombin time (PT)/INR** primarily assesses the integrity of the extrinsic (TF–VII) and common pathways.

5. Intrinsic pathway (contact activation pathway)

The intrinsic pathway is a powerful **amplification system** and is activated mainly on negatively charged surfaces (e.g., exposed collagen, polyphosphates, NETs).

Key steps:

1. **Contact activation**:
    - **Factor XII** binds to a negatively charged surface (collagen, kaolin, glass, etc.) and is converted to **XIIa**, aided by **prekallikrein** and **high-molecular-weight kininogen (HMWK)**.

2. **FXIIa → FXI → FXIa**.
3. **FXIa → FIX → FIXa** (Ca^{2+}-dependent).
4. Formation of **intrinsic tenase complex**:
    - **FIXa + FVIIIa + Ca^{2+} + phospholipid (on activated platelets)**.
    - This complex efficiently converts **FX → FXa**.
5. Positive feedback:
    - **Thrombin (IIa)** generated from early FXa activates:
        - **FV → FVa**
        - **FVIII → FVIIIa**
        - **FXI → FXIa**
        - Platelets themselves
    - This feedback loop produces a large burst of thrombin, dramatically amplifying coagulation.[5][6]

Clinical: The **activated partial thromboplastin time (aPTT)** primarily assesses intrinsic (XII, XI, IX, VIII) and common pathways.

6. Common pathway: from factor X to fibrin

Once **FXa** is generated (by either pathway), the common pathway proceeds:

6.1 Prothrombinase complex

- Components: **FXa + FVa + Ca^{2+} + phospholipid surface**.
- Converts **prothrombin (FII) → thrombin (FIIa)** very efficiently.
- FVa serves as a cofactor that massively increases FXa activity.

6.2 Thrombin's central roles

Thrombin is the key effector:

- **Converts fibrinogen (FI) → fibrin monomers** by cleaving fibrinopeptides A and B.
- Activates **FXIII → FXIIIa**, which **cross-links fibrin** (ε-(γ-glutamyl)-lysine bonds), stabilizing the clot.
- Strongly **activates platelets** via PAR receptors, promoting further aggregation and PS exposure.
- Amplifies the cascade (as above) by activating FV, FVIII, and FXI.
- Via thrombomodulin on endothelium, activates **protein C**, initiating anticoagulant feedback

6.3 Fibrin polymerization and stable clot

- **Fibrin monomers** spontaneously polymerize into a **fibrin mesh**, trapping RBCs, WBCs, and platelets.
- **FXIIIa** covalently cross-links fibrin strands, increasing mechanical strength and resistance to fibrinolysis.
- Platelet cytoskeletal contraction (actin-myosin) pulls on fibrin strands → **clot retraction**, shrinking the clot and drawing wound edges together.

7. Role of calcium, phospholipids, and vitamin K

7.1 Calcium (factor IV)

- Required as **Ca^{2+} bridges** between γ-carboxyglutamate residues on vitamin K-dependent factors and negatively charged phospholipids.
- Essential for assembly of **tenase** and **prothrombinase** complexes and for several activation steps (IX→IXa, X→Xa, II→IIa).

7.2 Phospholipid surfaces

- Activated platelets externalize **phosphatidylserine (PS)**, providing:
  - An **anchoring platform** for complexes (VIIIa–IXa; Va–Xa).
  - Local high concentration of enzymes and substrates → large kinetic advantage and spatial restriction of clotting to the site of injury.

7.3 Vitamin K-dependent factors

- Procoagulant: **II (prothrombin), VII, IX, X**.
- Anticoagulant/regulatory: **protein C, protein S**, **protein Z**.
- Vitamin K is required in the liver for **γ-carboxylation** of glutamate residues, generating **Gla domains** that bind Ca^{2+} and membranes.
- Warfarin and related anticoagulants inhibit **vitamin K epoxide reductase**, impairing this modification and thus **reducing the activity** of these factors.

8. Regulation: preventing excessive clotting

Normal hemostasis requires strong **negative regulation** to prevent pathological thrombosis.

Key natural anticoagulant systems:

8.1 Antithrombin (AT)

- A serine protease inhibitor (serpin) that **inactivates thrombin (IIa), IXa, Xa, XIa, XIIa**.
- Activity is markedly enhanced by **heparin** and heparan sulfate on endothelial cells, explaining the clinical use of heparin.

8.2 Protein C–protein S system

- On intact endothelium, thrombin binds **thrombomodulin**, changing its specificity.
- Thrombin–thrombomodulin complex activates **protein C → activated protein C (APC)**.
- **APC + protein S** inactivate **FVa and FVIIIa**, shutting down the tenase and prothrombinase complexes.
- This is a crucial negative feedback that focuses clotting on injured, not intact, endothelium.

8.3 Tissue factor pathway inhibitor (TFPI)

- Produced mainly by endothelium.
- Inhibits:
  - FXa directly.
  - The **TF–FVIIa–FXa complex**, thus down-regulating the **extrinsic pathway** once sufficient FXa has been generated.

9. Fibrinolysis: removal of the clot

After vessel repair, the clot must be removed to restore normal blood flow.

9.1 Activation of plasmin

- Circulating **plasminogen** is incorporated into the developing clot.
- Endothelial cells release **tissue plasminogen activator (tPA)**; monocytes and other cells can secrete **urokinase-type PA (uPA)**.
- On fibrin surfaces, tPA converts **plasminogen → plasmin**, a serine protease that:
  - Degrades fibrin into **fibrin degradation products (FDPs, including D-dimers)**.

9.2 Regulation of fibrinolysis

- **Plasminogen activator inhibitor-1 (PAI-1)**:
  - Released from activated platelets and endothelium.
  - Inhibits tPA and uPA, restricting fibrinolysis to appropriate times/locations.
- **α_2-antiplasmin**:
  - Inhibits free plasmin in plasma, preventing systemic fibrinogen degradation.

- Cross-linked fibrin (via FXIIIa) is more resistant to plasmin; this ensures that only stabilized clots persist long enough for repair.

10. Summary

It can be summarized as:

1. **Injury** → loss of endothelial antithrombotic factors, exposure of TF, collagen, and vWF.
2. **Primary hemostasis**:
    - Platelet **adhesion** (vWF–GPIb, collagen receptors).
    - Platelet **activation** (shape change, degranulation, TXA_2, PS exposure).
    - Platelet **aggregation** via GPIIb/IIIa and fibrinogen → **primary plug**.
3. **Secondary hemostasis**:
    - **Extrinsic TF–FVIIa** complex rapidly activates FX (and FIX).
    - **Intrinsic contact pathway** (XII–XI–IX–VIII) builds intrinsic tenase on platelet PS, massively activating FX.
    - **Common pathway**: FXa–FVa (prothrombinase) → thrombin → fibrin + FXIIIa cross-linking → **stable fibrin-platelet clot**.
4. **Regulation and resolution**:
    - Antithrombin, TFPI, and protein C/S prevent over-propagation of the clot.
    - Fibrinolysis (tPA → plasmin) gradually degrades fibrin once healing is sufficient.