**Hemostasis:** Prevention of blood loss is called Hemostasis.

**Coagulation/Clotting:** is the process in which blood looses its fluidity & becomes jelly like mass.

**Clot:** is a mesh of thin fibrils entangling the blood cells. These fibrils are made of fibrin.
Whenever a vessel is ruptured or severed, **hemostasis** is achieved by several mechanisms:

- Vascular constriction
- Formation of platelet plug
- Formation of a blood clot as a result of blood coagulation
- Growth of fibrous tissue into the blood clot to close the hole in vessel permanently
1. **Vascular constriction/ vascular spasm**

Spasm results from:
- Local myogenic response
  - smooth muscle contraction
- Local Autacoid factors from traumatized tissues and platelets
  - Thromboxane A2 from platelets
- Nervous reflexes like pain or other sensory impulses

This will last for minutes to hours during which time, platelet plug formation and coagulation can occur.
Platelets

- Minute discs 1-4 micrometer in diameter
- Formed from megakaryocytes in bone marrow or in blood
- 150,000 to 300,000 per microliter
- No nucleus, can not reproduce
- Half life is 8 to 12 days
Constituents of cytoplasm:

- Contractile proteins: Actin, myosin and thrombosthenine
- Residuals of Golgi apparatus & endoplasmic reticulum (synthesize enzymes and stores calcium)
- Mitochondria & enzyme systems that form ATP and ADP
- Prostaglandins
- Fibrin stabilizing factor
- Growth factor
- Three types of granules:
  - lysosomes
  - Dense granules having calcium, serotonin, ADP, ATP
  - Alpha granules having fibronectin, PDGF
Membrane properties:

- Coat of glycoproteins that repulses adherence to normal endothelium and cause adherence to injured endothelial cells and collagen
- Phospholipids activate multiple stages in blood clotting process
Platelet plug formation:
- On coming in contact with damaged vascular surface especially collagen, platelets change their characteristics
  - Swell, change to irregular forms with pseudopods
  - Contractile proteins contract forcefully
  - Release of granules containing multiple active factors, ADP & Thromoxane A2, activate other platelets
  - Become sticky, attached to von Willebrand factor
- Form a loose platelet plug
- Significance
PLATELET PLUG

Close minute ruptures in very small blood vessels thousands of times daily
1. Platelets adhere to and are activated by exposed collagen at the site of vessel injury.
3. These chemical messengers work together to activate other platelets passing by.
4. Newly activated platelets aggregate onto growing platelet plug and release even more platelet-attracting chemicals.
5. Normal (uninjured) endothelium releases prostacyclin and nitric oxide, which inhibit platelet aggregation, so platelet plug is confined to site of injury.

**Figure 2:** Platelet aggregation at the site of vessel injury.
**Formation of blood clot or Coagulation**

**Activation time**
- 15 to 20 sec in severe trauma
- 1 to 2 min in case of minor trauma
- Within 3 to 6 min bleeding stops & after 20 min to 1 hr clot retracts.

**Depends upon**
- Vascular spasm
- Platelet activation
- Blood clotting factors
Most of them are plasma proteins ($\beta$ globulin) formed in the liver.

Vitamin K-dependent clotting factors are:

- II, VII, IX, X

Most of them are present as proenzymes (inactive)

Once activated, it induces a cascade reaction.

---

### Coagulation (Clotting) Factors

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<th>Synonyms</th>
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<td>Tissue factor</td>
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Procoagulants and Anticoagulants

Procoagulants are substances that promote coagulation
Anticoagulants are substances that inhibit coagulation

• Whether the blood will coagulate or not, depends on the balance between the two groups
• Anticoagulants normally predominate so the blood does not coagulate while circulating in blood vessels
Main steps for clotting

1. In response to damage to the vessel or blood itself, a complex cascade of chemical reactions occur involving more than a dozen coagulation factors resulting in complex of activated substances called PROTHROMBIN ACTIVATOR
2. It catalyzes conversion of prothrombin to thrombin
3. It acts as enzyme to convert fibrinogen into fibrin fibers
How the prothrombin activator is formed?

- Trauma to the vascular wall and adjacent tissues
- Trauma to the blood
- Contact of blood with damaged endothelial cells or collagen

Initiate two pathways that interact constantly with each other

- **Extrinsic pathway**
  - Trauma to the vascular wall & surrounding tissue

- **Intrinsic pathway**
  - Trauma to blood or blood comes in contact with collagen
    - **In vivo** - collagen
    - **In vitro** - glass that begins in the blood
Extrinsic pathway for initiating clotting

1. Tissue trauma → Tissue factor
2. VII → VIIa → X → Activated X (Xa) → Prothrombin Activator → Thrombin
3. Platelet phospholipids → Prothrombin → Thrombin

Ca++ plays a role in each step.
Intrinsic pathway for initiating blood clotting.
Extrinsic Pathway

Damage to tissue outside the vessel

Tissue Thromboplastin

Inactive Factor X

Activated Factor X

Prothrombin

Thrombin

Fibrinogen

Fibrin

Factor XIII

Blood Clot

Intrinsic Pathway

Damage to the blood vessel

Cascade of clotting factors

Activated Factor X

Prothrombin

Thrombin

Fibrinogen

Fibrin

Factor XIII

Blood Clot
(a) The coagulation phase
Conversion of prothrombin to thrombin

Prothrombin activator in the presence of sufficient amounts of ionic calcium(Ca++) causes conversion of prothrombin to thrombin
Figure 37-2. Schema for conversion of prothrombin to thrombin and polymerization of fibrinogen to form fibrin fibers.
Prothrombin:
• A plasma protein, alpha 2 globulin
• Molecular weight 68700
• Normal conc 15 mg/dl
• Unstable protein that can split easily into smaller compounds
• Continuously formed by liver
• Used throughout the body for blood clotting
• Depends on vitamin K for normal activation

Thrombin:
• Smaller compound formed by splitting of prothrombin
• Molecular weight 33700
• Protein enzyme with weak proteolytic capabilities
Action of Thrombin on Fibrinogen to form Fibrin
- Formation of Clot

Fibrinogen:
• High molecular weight protein (340,000) present in plasma 100 to 700mg/dl
• Synthesized in liver
• It is one of the essential factor required for clotting
• Very little leak from capillaries to interstitial space under normal conditions
Fibrinogen Molecule → Thrombin → Fibrin monomer

- Removal of four LMW peptides

Fibrin fibers with covalent bonds & multiple cross linkages → Fibrin stabilizing factor

- Activated by thrombin

Polymerization

Fibrin stabilizing factor → Long fibrin fibers

- Weak hydrogen bonding
(a) Vascular spasm
(b) Platelet plug formation
(c) Blood clotting
Blood clot

The clot is composed of a meshwork of fibrin fibers running in all directions and entrapping blood cells, platelets and plasma. The fibrin fibers also adhere to damaged surfaces of blood vessels, vascular openings and prevents blood loss.
Role of thrombin

1. Thrombin, a component of the clotting cascade, plays multiple roles in hemostasis:
   - 1a. Stimulates conversion of fibrinogen to fibrin
   - 1b. Activates factor stabilizing fibrin meshwork of clot
   - 1c. Enhances activation of more prothrombin into thrombin through positive feedback
   - 1d. Enhances platelet aggregation

2. Through positive feedback, aggregated platelets secrete PF3, which stimulates clotting cascade that results in thrombin activation.

**FIGURE 11-13** Roles of thrombin in hemostasis.
Compare and contrast extrinsic and intrinsic pathways

• Whether intrinsic or extrinsic leads to formation of prothrombin activator.
• Both systems operates by positive feedback mechanism.
• Both systems starts simultaneously.
• Calcium is needed in both the mechanisms.

Step 2 in extrinsic and step 3 in intrinsic pathway.
Compare and Contrast extrinsic and intrinsic pathways

- Response of **extrinsic pathway is rapid**, explosive, within seconds while the response time of **intrinsic pathway is slow**, takes 1-6 min.

- **Tissue factor** initiates extrinsic pathway whereas contact of **factor XII & platelets** with collagen initiates intrinsic pathway

- Both the pathways **meet at one step i-e**
  
  $$X \text{ to } Xa.$$
Clot retraction

- Clot begins to contract within a few minutes of its formation
- Expresses most fluid in 20 to 60 minutes - Serum
- Edges of broken blood vessel are pulled together, contributing in Hemostasis.

Factors that cause clot retraction:
- Platelets are essential for clot retraction. Release Fibrin stabilizing factor
  Contribute directly by contraction of thrombosthenin, actin & myosin molecules
- Thrombin
- Calcium ions
Role of Ca$^{+2}$ in blood clotting

Ca$^{+2}$ required for acceleration of most of blood clotting reactions

1. Absence of Ca$^{2+}$ prevent blood clotting by the 2 pathways.
2. Citrate and oxalate salts (Ca$^{2+}$ precipitating agents) can be used as in vitro anticoagulants.
Positive feedback of clot formation

Once a clot has started to develop, it normally extends within minutes into surrounding blood - the clot initiates a positive feedback to promote more clotting.

- Direct proteolytic action of thrombin on prothrombin
- Acceleration of the actions of factors VIII, IX, X, XI and XII, aggregation of platelets by thrombin
How clotting is prevented in the normal vascular system???

**Endothelial surface factors**

- Smoothness of the endothelial cell surface which prevent contact activation of intrinsic clotting system
- Layer of glycocalyx (mucopolysaccaride) on the endothelium
- A protein bound with endothelial membrane, **thrombomodulin** which binds thrombin

**Thrombin-thrombomodulin complex** activates protein C that acts as an Anticoagulant by inactivating activated factors V and VIII

- Slow the clotting process by removing thrombin
Anticoagulants in blood

- **Fibrin fibers** 80 to 90% of thrombin becomes adsorbed to fibrin prevent the spread of thrombin to the remaining blood, prevents excessive Spread of the clot

- **Antithrombin III or antithrombin-heparin cofactor** an alpha globulin
  If not adsorbed on fibrin, thrombin combines with antithrombin III, It blocks the effect of thrombin on fibrinogen, inactivates thrombin in next 12 to 20 minutes

- **Heparin (secreted by mast cells & basophils)**
  - It increases the effectiveness of antithrombin III for removing thrombin
  - This complex also remove other activated coagulation factors like XII, XI, X and IX
Lysis of clot

Activation of plasminogen to form plasmin
- In clot, large amount of plasminogen (Profibrinolysin) is trapped along with other plasma proteins
- Tissue plasminogen activator (t-PA), released slowly by injured tissues and vascular endothelium caused activation of plasminogen to become plasmin.
  - Plasmin digests fibrin fibers and some other protein coagulants, removes unnecessary blood clot
t-PA

Plasminogen $\rightarrow$ plasmin

**lysis of clot by**
inactivating factors **I, II, V, VIII & XII**

- **t-PA** (Tissue Plasminogen Activator) used in MI and stroke for clot dissolution.
- **Streptokinase**, also a fibrinolyisin used for clot dissolution.
Fibrinolytic system

In plasma

Plasminogen (profibrinolysin) → Plasmin (fibrinolysin) → Fibrin → Fibrin degradation products (FDPs)

Plasminogen activator (tPA Urokinase) → Plasmin → Fibrin → Fibrin degradation products (FDPs)

streptokinase → Plasminogen → Plasmin → Fibrin → Fibrin degradation products (FDPs)
Significance of plasmin system

• It removes minute clots from millions of tiny peripheral vessels that would become occluded if this system is not there
• Many small blood vessels are reopened by this mechanism.
Bleeding disorders
Vitamin K deficiency

Vitamin K is required for the synthesis of five clotting factors: Factors II, VII, IX, X and protein C

- It is essential factor to a liver carboxylase that adds a carboxyl group to glutamic acid residues on these clotting factors
- Vitamin K itself becomes oxidized & inactivated
- It is activated back by Vitamin K epoxide reductase complex I (VKOR c1)
- In the absence of active vitamin K, serious bleeding tendencies can develop
Vitamin K deficiency can occur in
- Poor absorption of fats from GIT
- Obstruction of bile ducts
- Liver disease

Vitamin K injection is given to the patients of liver disease before surgery
Hemophilia

An X- linked bleeding disorder that occurs exclusively in males.

Types:

**Hemophilia A or classic Hemophilia:** 85% cases - deficiency or abnormality of factor VIII (smaller component)

**Hemophilia B or Christmas Disease:** 15% cases – deficiency of factor IX

- Both these factors are transmitted genetically by way of female chromosome.
- A woman can be a carrier if one of the two X chromosomes is defected.
Features of hemophilia:

Severe and prolonged bleeding can start after a minor or unnoticeable trauma.
Hemophilia
Treatment

• Injection of purified factor VIII
• Increasing use of recombinant factor VIII
**Thrombocytopenia**

The presence of very low numbers of platelets in circulating blood. The bleeding occurs from small venules and capillaries and not from large blood vessels.

- Bleeding starts when platelet count falls below 50,000/µl
- Level as low as 10,000 is lethal

**Thrombocytopenic purpura:**

Small punctate hemorrhages occur throughout the body. The skin of the patient displays many small purplish blotches.
Idiopathic thrombocytopenic purpura (ITP)
If thrombocytopenia is due to unknown cause, it is called ITP.
• Antibodies are formed and react against platelets to destroy them.

Secondary thrombocytopenia:
Can occur due to:
• Some viral diseases
• Drugs
• Hypersplenism

Treatment:
• Fresh whole blood transfusion
• Splenectomy
Thromboembolic conditions

Thrombus: An abnormal clot that develops in a blood vessel is called thrombus

Emboli: Due to blood flow, the clot may break away from its attachment. Such freely flowing clots are called emboli.

Cause:
• Roughened endothelial surface (arteriosclerosis, infection, trauma)
• Very slow blood flow

Treatment: Use of tPA delivered through a catheter
Disseminated intravascular coagulation

If the clotting mechanism becomes activated in widespread areas of the circulation, condition is called disseminated intravascular coagulation (DIC).

**Cause:** Presence of large amount of traumatized or dying tissues in body that Release tissue factor e.g septicemia
Tests for coagulation

- **Bleeding time:** Time taken by the blood to stop oozing from a cut surface is called bleeding time. It is usually 1 to 6 minutes. Prolonged bleeding time results from lack of any of the clotting factors, especially by lack of platelets.

- **Clotting time:** The time taken by the blood to clot is called clotting time. It varies greatly depending on the method used. Can be 6 to 10 minutes.
Prothrombin time PT:
It is a measure of the extrinsic pathway of blood clotting & is used to Monitor the effectiveness of oral anticoagulant like warfarin. It is 12 to 14 seconds.

Prolonged PT:
• Liver disease
• Vitamin K deficiency
• Isolated clotting factor deficiencies like factors I,II,V & X
Activated partial thromboplastin time APTT:
This test is a general coagulation screening test which gives information about the intrinsic coagulation pathway (factors XII, XI, IX, VIII, X, V, II & I)
Anticoagulants for clinical use

**Heparin:** Injection of small quantity can increase clotting time from 6 minutes to 30 minutes thus immediately preventing or slowing thromboembolic condition.

**Warfarin**

**Oral anticoagulant** - It decreases the available active form of vitamin K by inhibiting the enzyme VKORc1.

When given to patient, amount of active prothrombin, VII, IX & X, all formed by the liver begin to fall.
Thank You