Basic Biomedical Sciences 1

Lecture 2: Hemostasis

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HEMOSTASIS

HEMO

- **Hemo** = blood

STASIS

- **stasis** = stoppage of flow of bodily fluids

HEMOSTASIS

- **Hemostasis** = stopping blood from exiting the bloodstream
  - Process by which blood clots are formed at the site of injury
- If hemostasis is deranged, broadly classified in two groups of disorders:
  1. Hemorrhagic disorders: characterized by excessive bleeding. Hemostatic mechanisms are blunted or insufficient to prevent blood loss.
     - i.e. Hemophilia or factor deficiencies in blood clotting where there are insufficient mechanisms
  2. Thrombotic disorders: Blood clots form within intact blood vessels or within the chambers of the heart.
     - I.e. pulmonary embolism and myocardial infarction
- Sometimes division between bleeding and thrombotic disorders are not so clear cut
  - I.e. generalized activation of clotting sometimes **paradoxically** produces bleeding due to consumption of coagulation factors (Disseminated Intravascular Coagulation)

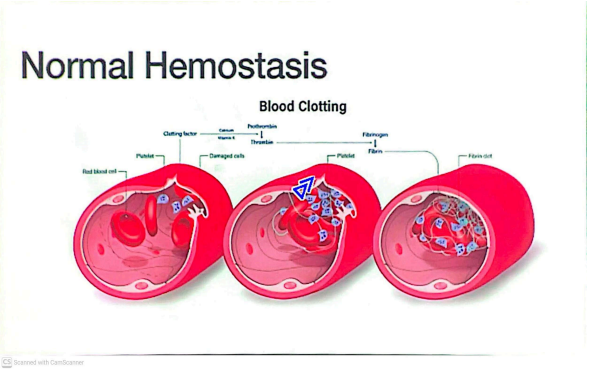


Figure 1: Normal Hemostasis

- The blood vessel got damaged and is exposed to the surrounding tissues. The platelet (blue structures) together with clotting factors, cofactors, vitamin K that would mark the coagulation cascade, ultimately resulting in fibrinogen becoming fibrin. These will form primary clots or platelet plug and once you have fibrin you now have fibrin clot

NORMAL HEMOSTASIS

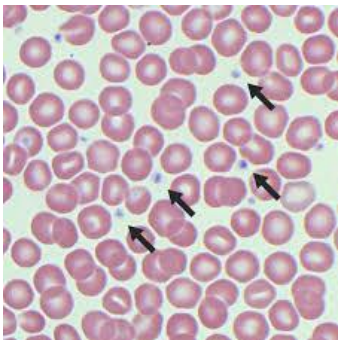
- A process involving platelets, clotting factors, and endothelium that occurs at the site of vascular injury and culminates in formation of blood clot which serves to prevent or limit extent of bleeding.

SEQUENTIAL EVENTS LEADING TO HEMOSTASIS

- **Vasoconstriction**: Occurs immediately and serves to reduce blood flow to the injured area.
  - First response and temporary
  - Due to smooth muscles in the tunica media of the blood vessel to contract. Nerves and factors that regulate or control vasoconstriction and vasodilation.
  - Serves to slow down the blood to prevent blood loss.
  - Temporary; if there is no platelet plug formation, it will go back and blood will be lost

PRIMARY HEMOSTASIS

- **formation of platelet plug**: Disruption of endothelium exposes subendothelium von Willebrand Factor and collagen which promote platelet adherence and activation meaning that they will change their morphology and their granules. Instead of the normal disc shaped platelet you get a “sea urchin” shaped platelet because of its spiny processes and its purpose is to increase surface area so that it can release granules.
- There are two types of granules: Alpha granules and Dense granules



- Peripheral blood smear of what platelets look like under a microscope and their basophilic fragments

VON WILLEBRAND FACTOR (vWF)

- **von Willebrand Factor**
  - Glycoprotein that helps platelet stick to injured vessel walls to form a primary platelet plug
  - Also carries **factor VIII** for coagulation
  - Produced by endothelial cells and megakaryocytes
    - stored in **WEBEL-PALADE BODIES** in the endothelium
    - Alpha granules of platelets
  - Binds to receptor **GP Ib (Glycoprotein Ib)** on platelets so in that way they can stick to each other.

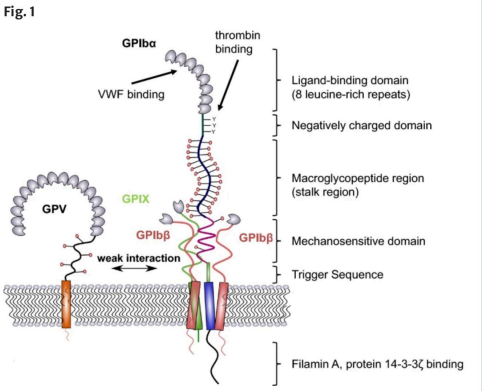


Figure 2: Glycoprotein Ib

- Found in platelets and endothelium and serves as a glue; it will adhere the platelets together (primary hemostasis)

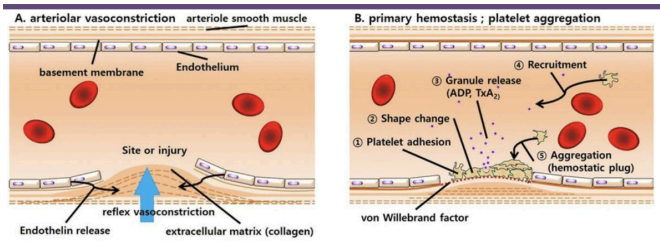


Figure 3: Vasoconstriction ; Primary Hemostasis ; Platelet Aggregation

Here's the diagram, the first step on the left shows that the blood vessel is injured and the immediate reaction is vasoconstriction slowing the blood flow. Because the endothelium is disrupted, it exposes the underlying tissues to collagen and tissue factors. When the von Willebrand factor (vWF) will be released and exposed, it will bind to receptor GP Ib (1) and that way the platelets can now attach to the vWF then it will undergo platelet activation which is basically a change in morphology and releasing of granules which is called **platelet aggregation** (2) and (3)**.** It will release substances such as ADP and thromboxanes A2 which will promote more platelet recruitment and aggregation (positive feedback mechanism). Once all the platelets are joined together, that is the formation of **platelet plug formation.**

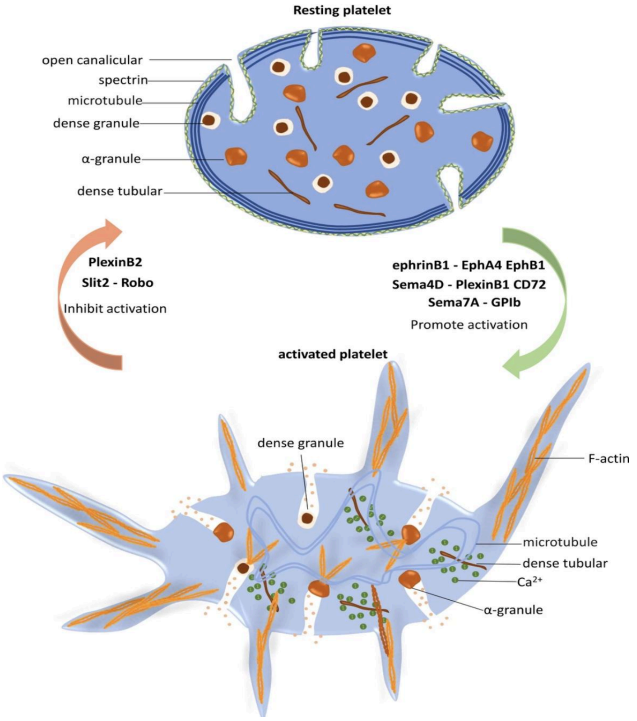


Figure 4: Neuronal activation

When platelets become activated, they exposed phospholipid surfaces that bind to calcium where coagulation cascade nucleate.

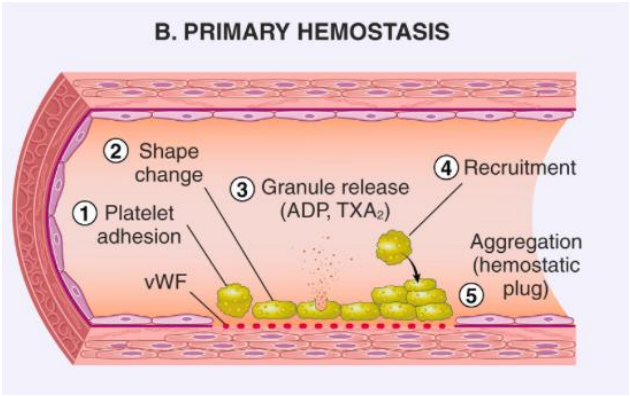


Figure 5: Platelet Plug Formation

The illustration shows the formation of platelet plug; adhesion, activation, recruitment and formation of your plug which is temporary wherein it needs to be stabilized through the production of fibrin. Fibrin is like a meshwork acting as a thick chain of rope that surrounds the platelet plug.

SECONDARY HEMOSTASIS

- deposition of fibrin: Injury exposes tissue factor at sites of injury It binds and activates factor VII setting in motion the coagulation cascade that culminates in **thrombin** generation. Thrombin cleaves through fibrinogen into insoluble fibrin creating a **fibrin mesh work**. This process consolidates the initial platelet plug

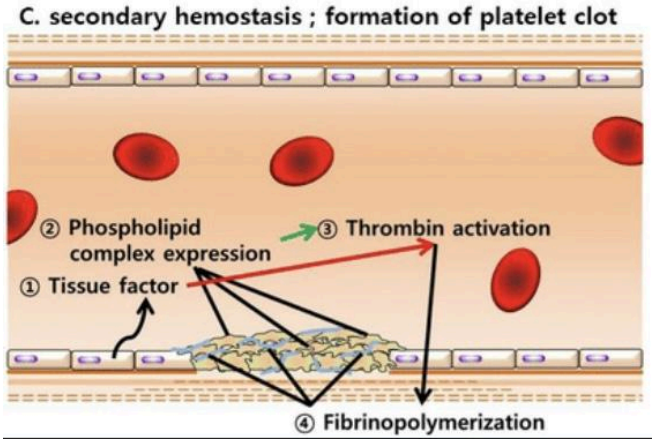


Figure 6: Secondary Hemostasis

The illustration shows secondary hemostasis. The dark-blue lines signify the fibrin. Tissue factor is exposed initiating the coagulation cascade. Phospholipids are produced from the platelets which are the nucleation sites for coagulation factors. Thrombin is activated which will result to fibrin becoming polymerized or insoluble (fibrinopolymerization)

- Secondary plug has **fibrin**
- Initial plugs are just **platelets**

CLOT STABILIZATION AND RESORPTION

- polymerized fibrin and platelet aggregates undergo contraction to form a solid permanent plug. The counter regulatory mechanisms (tissue plasminogen activator, made by endothelial cells) are activated that limit clotting to the site of injury and eventually lead to clot resorption and tissue repair.

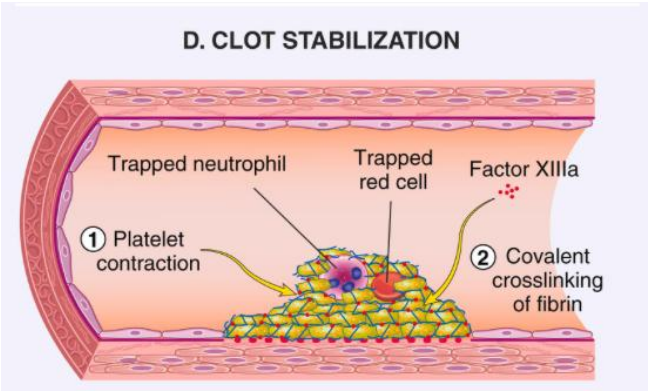


Figure 7: Clot Stabilization

ENDOTHELIAL CELLS

- Central regulators of hemostasis. It depends on the state of endothelium on how to prevent excessive blood clotting.
  - If it is intact: blocks the collagen and tissue factors; platelets cannot reach the tissue factor vWF because they are already stored inside the cell and they are physically blocked by the cell.
  - If it is damaged: blood clot formation is favored

- Balance between antithrombotic and prothrombotic activities determine if thrombus forms and grows or dissolution occurs
- Normal endothelial express anticoagulant factors
- After injury or activation, endothelial cells undergo for coagulation
- Aside from trauma, endothelial activation can be done by microbial pathogens, hemodynamic factors and pro-inflammatory mediators.

3 MAJOR COMPONENTS

PLATELETS

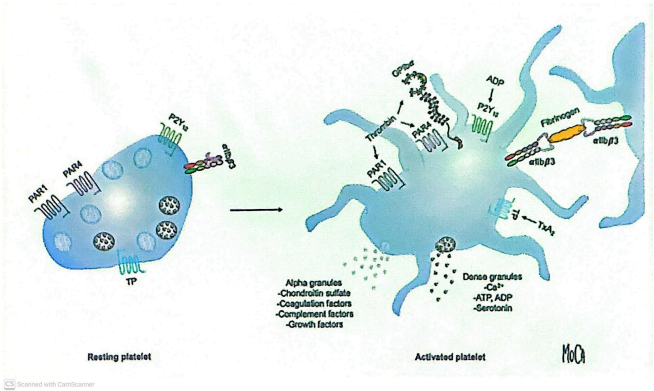


Figure 8: Platelets

- Platelets originate from megakaryocytes from the bone marrow; they are fragments of megakaryocytes.
  - Megakaryocytes give rise to platelets: pinch off the cytoplasm
- Anucleated; dense ovoid structures in peripheral blood smear
- When they come in contact with underlying epithelium, they undergo platelet activation; they change their morphology(sea urchin; spiny processes) and release granules
- Sea urchin morphology - increase surface area in order to release granules
- Platelets play a critical role in forming the primary plug and binds coagulation factors
- Granules of Platelets

TYPES OF GRANULES

- **Types of granules**
  - Alpha Granules
  - Dense Granules

Alpha Granules

- **Alpha Granules:**
  - Molecules For adhesions: P selectin
  - Proteins for coagulation: fibrinogen, coagulations factors (V, XI, vWF)
  - Factors for wound healing: fibronectin, platelet factor 4, PDGF, TGD-Beta

Dense Granules

- **Dense Granules**
  - ADP, ATP, Ionized calcium, serotonin, epinephrine

"Then you have your dense granules which has ATP and other substances"

CELL MEMBRANE OF PLATELETS

- **Cell Membrane of Platelets** - contain several glycoprotein receptors important for hemostasis
  - Gp Ib (vWF)
  - Gp VI and Gp Ia/IIa complex (collagen)
  - GpIIb/ IIIa complex (fibrinogen)

"Then, on the membrane of the platelets there are various receptors. We talked about one of them, the Gp1b for vWF but here's also a receptor for collagen and fibrinogen"

- **What happens when platelets encounter sites of vessel injury**

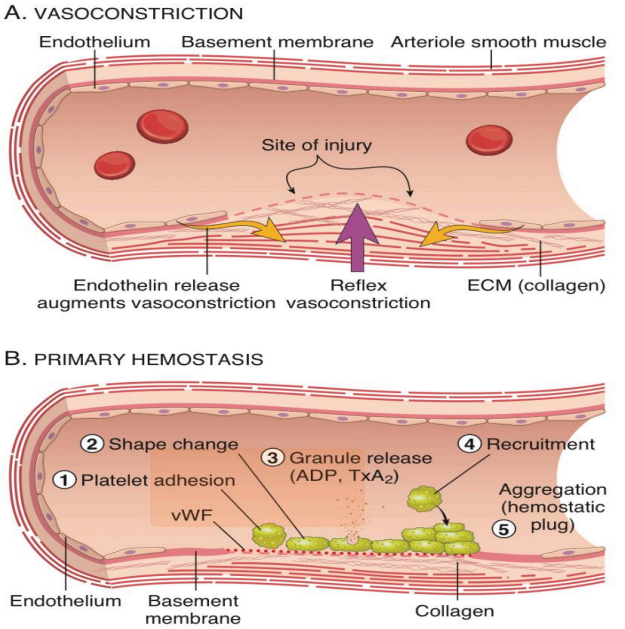


Figure 9: Vasoconstriction ; Primary Hemostasis

  - Vasoconstriction
  - Damaged vessel exposes subendothelial connective tissue (vWF and collagen)
  - Platelets come to contact which causes a sequence of reactions to form a platelet plug

"So what happens to the platelets when they encounter sites of injury. The first step is always vasoconstriction but before vasoconstriction, well, first is injury then vasoconstriction, so the damaged blood vessels will expose the vWF, platelets come in contact and become activated."

SEQUENCE OF PLATELET PLUG FORMATION

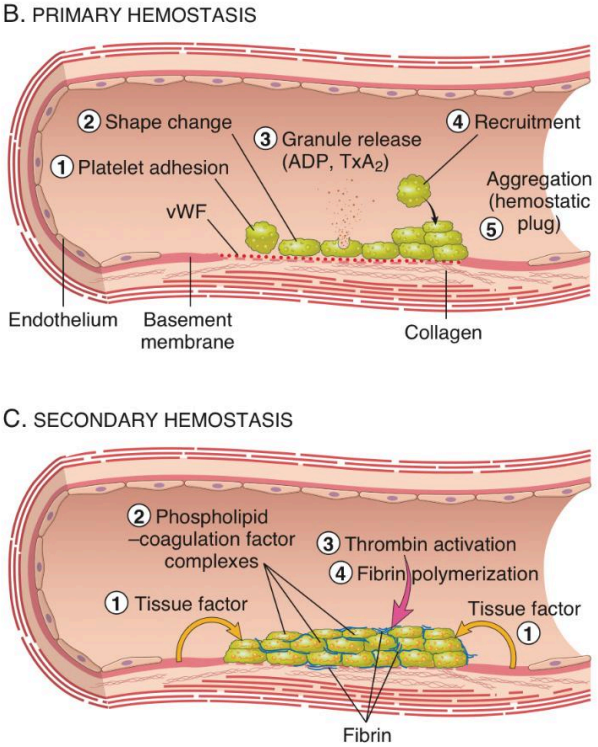


Figure 10: Primary Hemostasis ; Secondary Hemostasis

  - Platelet adhesion
  - Platelet change shape
  - Secretion of granules
  - Platelet aggregation

"So here's the special steps: adhesion, shape change, secretion of granules, and aggregation. Collectively, this is platelet activation."

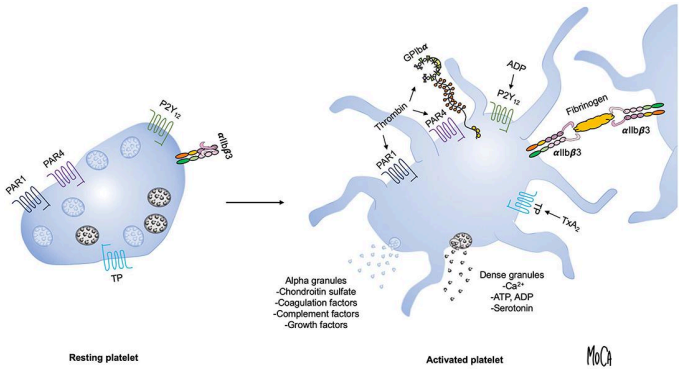


Figure 11: fibrinogen attached to αIIbβ3

"So you see in the diagram that I showed you earlier and I'm showing it to you again. Here is the fibrinogen attached to its αIIbβ3. Note this, you have ATP here will also act as positive feedback and will act on this receptor, the P2Y12 receptor. Thromboxane is important, it's also produced by the platelet by cyclooxygenase. If you are familiar with aspirin, aspirin inhibits cyclooxygenase. Therefore, inhibiting thromboxane formation. Therefore, inhibiting platelet aggregation. That's how it is known as a blood thinner because of inhibition of cyclooxygenase. Then, thrombin itself also will activate platelets by its PAR1 receptor and PAR4 receptor."

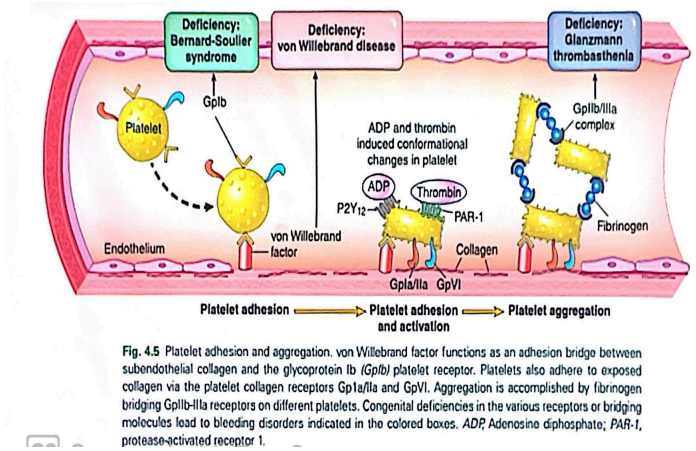


Fig. 4.5 Platelet adhesion and aggregation. von Willebrand factor functions as an adhesion bridge between subendothelial collagen and the glycoprotein Ib *(GpIb)* platelet receptor. Platelets also adhere to exposed collagen via the platelet collagen receptors Gp1a/IIa and GpVI. Aggregation is accomplished by fibrinogen bridging GpIIb-IIIa receptors on different platelets. Congenital deficiencies in the various receptors or bridging molecules lead to bleeding disorders indicated in the colored boxes. *ADP*, Adenosine diphosphate; *PAR-1*, protease-activated receptor 1.

Figure 12: Platelet adhesion ; activation ; aggregation

"You can see those vWF, that's the red little head sticking up, see how it is attached to the receptor, the GpIb. Then it will undergo conformational change/activation. It will release ADP and will self-activate and initiate the coagulation cascade which will reduce thrombin which will also activate more platelets and ultimately result in fibrinogen adhering several platelets together."

When a factor number has a letter "a" after it, it means it is in its activated form

- EXAMPLES.
  - Factor II: Prothrombin (inactive),
  - Factor IIa: Thrombin (active)

The extrinsic and intrinsic pathways will merge to initiate the common pathway. The most important factor in this pathway is **thrombin**.

THE COAGULATION CASCADE

It is a series of amplifying **enzymatic reactions** that lead to **deposition** of an insoluble **fibrin** clot.

- Mediators (factors) are mostly plasma proteins that circulate in inactive form
- Exception is tissue factor (factor III) which is found in cells (i.e. fibroblasts and pericytes) and is released only after tissue injury when exposed to blood.
- Coagulation factors are passed from one step to another
- Each step involves an **enzyme** (activated coagulation factors), **substrate** (inactivated proenzyme of a coagulation factor), and **cofactor**

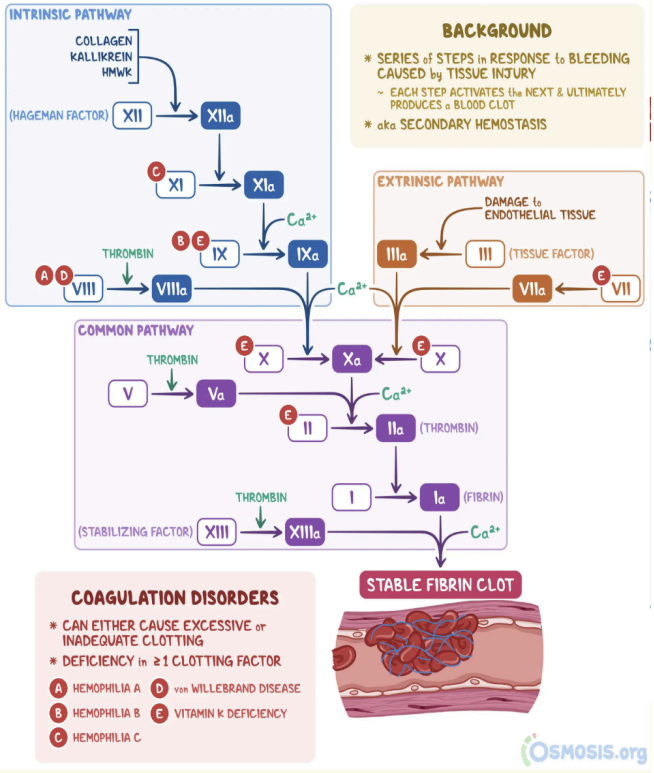


Figure 13: Coagulation Cascade

- Most of these factors are just circulating into the blood themselves, except for tissue factor, which is usually within the tissues.
- Ultimately, the **end result** of the coagulation cascade is to produce the **fibrin**, which will form the secondary clot and stabilize the clot.
- These factors are assembled within the surface of the platelets. The platelets have **phospholipids which** will also bind to **calcium.**
- If the **process** requires calcium, it will bind to a substance which is dependent on **vitamin K.**
  - If you have vitamin K **deficiency,** there is excessive bleeding because portions of the coagulation cascade cannot be completed. That's why vitamin K is given to the **newborns.**
- Factors assembly on phospholipids surface provided by activated platelets and depends on calcium (factor IV) which binds to gamma carboxylated glutamic acid present on **factor II, VII, IX, and X** (vitamin K-dependent)
  - Reactions that produce gamma carboxylated glutamic acid use **vitamin K** as a cofactor.

COAGULATION FACTORS

Name	Synonym
Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Tissue factor
Factor IV	Calcium
Factor V	Proaccelerin, labile factor

Factor VI	Old name of Factor Va
Factor VII	Proconvertin, stable factor
Factor VIII	Antihemophilic factor A
Factor IX	Antihemophilic factor B
Factor X	Stuart-Prower factor
Factor XI	Plasma thromboplastin anther
Factor XII	Hageman factor
Factor XIII	Fibrin-stabilizing factor

- Laboratory tests used to assess the pathways are divided into two:
  - **Extrinsic Pathway :** assessed by the **prothrombin time (PT)**.
  - **Intrinsic pathway**: assessed by **partial thromboplastin time (PTT)**
- Both of them will measure the **common pathway.**
- If we suspect a **deficiency** in one of the factors, we start off with these lab tests.
  - If **one** of them is **abnormal** and the other one is normal, you can probably assume that there's a deficiency in **either** the extrinsic or intrinsic
  - If **both** of them are **abnormal**, the deficiency is expected in the common pathway.
  - But nowadays, there are assays to measure each individual factor but they are a little bit expensive.

THROMBIN (FACTOR IIA)

- **The most important factor**
  - Effects of Thrombin
    - Conversion of fibrinogen to fibrin
    - Also acts as cofactors for activation of Factors V, VIII
    - Activates Factor XIII which cross links fibrin
  - Platelet Activation
    - Via activating PAR-1
  - Pro inflammatory Effects
    - Repair and angiogenesis
  - Anticoagulant
    - On intact epithelium it acts as anticoagulant

FACTORS THAT LIMIT COAGULATION

- **Dilution**
  - Blood flowing past injury washes out activated coagulation factors which are removed by liver
- Requirement for negatively charged phospholipids done by the platelets
- Factors expressed by notated endothelium adjacent to injury
- **Fibrinolytic Cascade**
  - Limits size of clot and later its dissolution
  - Enzyme **plasmin** which breaks fibrin and interferes with polymerization
- Coagulation cascades meet the phospholipids on the platelets.
- So when the platelets are activated, they will form the phospholipids on the surface, that's where the cascade will occur.
- The factors expressed by the normal endothelium exhibit **anticoagulatory effects**

FIBRINOLYTIC CASCADE

- **Fibrinolytic cascade**
  - **Fibrinolysis**: fibrin breakdown, it prevents the blood clot from forming
  - Broken down by **plasmin**
  - It interferes with the build up fibrin and interferes with polymerization

ENDOTHELIUM

- Endothelium is balanced between anticoagulant and pro coagulant activities, it will either promote or inhibit
  - **Damaged:** promote coagulation
  - **Intact:** inhibit coagulation
- Normal endothelium cells
  - Express factors that inhibit procoagulant activities of platelets and coagulation cells
  - Augment fibrinolysis
- Injured endothelium or endothelium exposed to pro inflammatory factors
  - Loose antithrombotic properties

ANTITHROMBOTIC PROPERTIES OF ENDOTHELIUM

- Platelet inhibitory effects
- Serves as a barrier that shields platelets from sub endothelial vWF and collagen

Secretion of inhibitory factors

- Prostacyclin: produced by COX 1
- Nitric oxide
- Adenosine diphosphotase: enzyme that breaks down ATP
- Thromodulin: works with endothelium protein C to inhibit thrombin
- Heparin like molecules: inhibits thrombin by activating anti-thrombin

Fibrinolytic factors

- Normal endothelial cells synthesizes t-PA which is importantly in fibrinolytic pathway
- t-PA is the most important activator of plasminogen which forms plasmin