Physiology

Sheet: 7

Lecture title: General overview of hemostatic process (Blood coagulation)

Date: 9/3/2022

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Edited by: Academic Team leaders

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بسم الله الرحمن الرحيم

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فيديو مهم قبل بداية المحاضرة بسهّل كثير شغلات بشرح ٨٠٪ من المحاضرة

Lecture 6 and 7
General overview of hemostatic process (Blood coagulation)

- ✓Understand the process and stages (cascade) of blood coagulation and its significance.
- ✓List and understand the role of factors involved in blood coagulation.
- ✓Understand the role of serine proteases in the cascade of blood coagulation.
- ✓Understand the intrinsic and extrinsic Pathways of blood clot
- ✓Understand the cause of excessive bleeding
- ✓Understand bleeding time, clotting time and prothrombin time

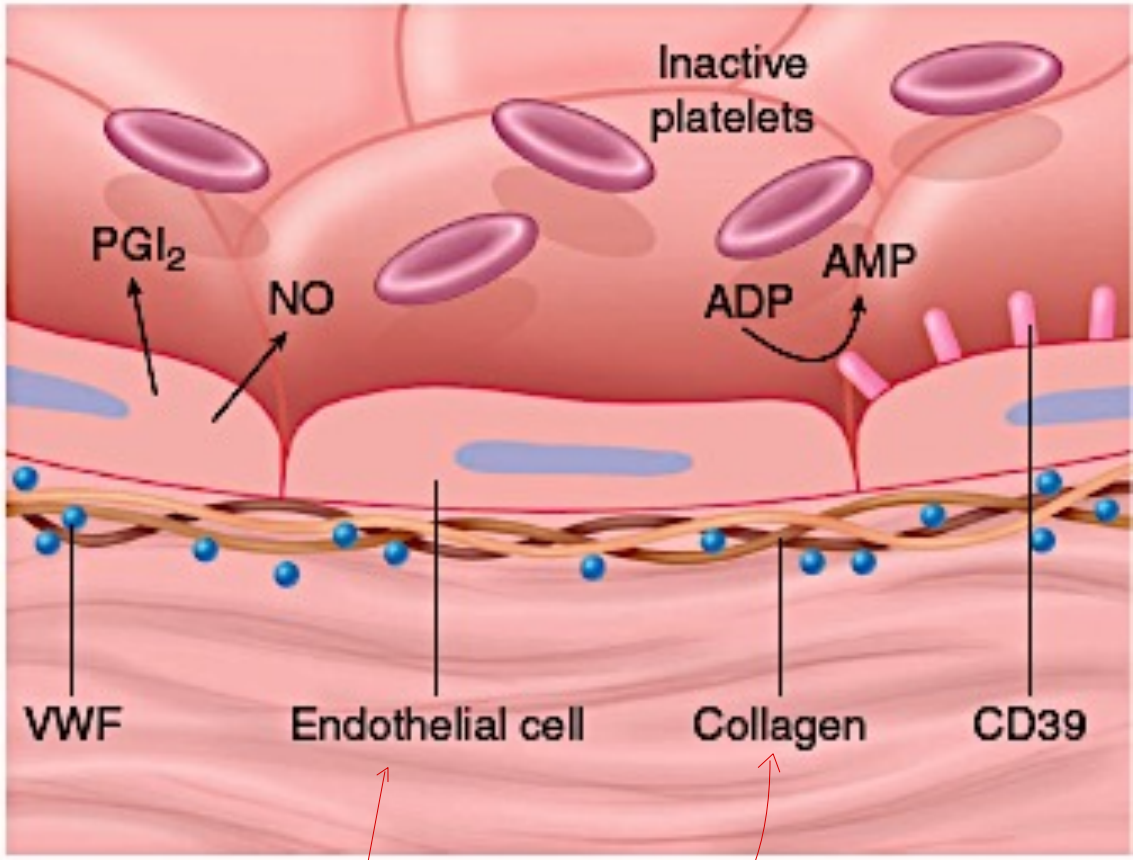
Hemostasis

- It is the process of forming clots (coagulation) in the walls of damaged blood vessels and preventing blood loss while maintaining blood in a fluid state within the vascular system
- Damage exposes collagen fibers to blood, producing:

a. Vasoconstriction→ reduces blood flow, result from (1) local myogenic spasm, (2)local autacoid factors (3) nervous reflexes
b. Formation of platelet plug (*primary hemostasis):* small cut sealed by a platelet plug rather than by a blood clot
c. Formation of a blood clot (*Secondary hemostasis*): damaged endothelial cover of blood vessels
d. Formation of fibrin protein web to close the hole in the vessel permanently

Intact endothelium protective mechanisms:

- Separate the blood from collagen
- Secretes prostacyclin *(PGI_2)* and nitric oxide, which:
  1)Vasodilate
  2)Inhibit platelet aggregation
- Secrets CD39 Enzyme, which breaks down ADP into AMP and P_i to inhibit platelet aggregation further
- Damaged endothelium exposes collagen



مهم جدا نعرف الوضع الطبيعي قبل ما يصير شق في الأوعية الدموية و هذا السلايد بشرح كيف بكون الدم منفصل عن الكولاجين الموجود اسفل الخلايا المبطنة للأوعية الدموية.

Damaged endothelium exposes collagen

1)Platelets bind to collagen.

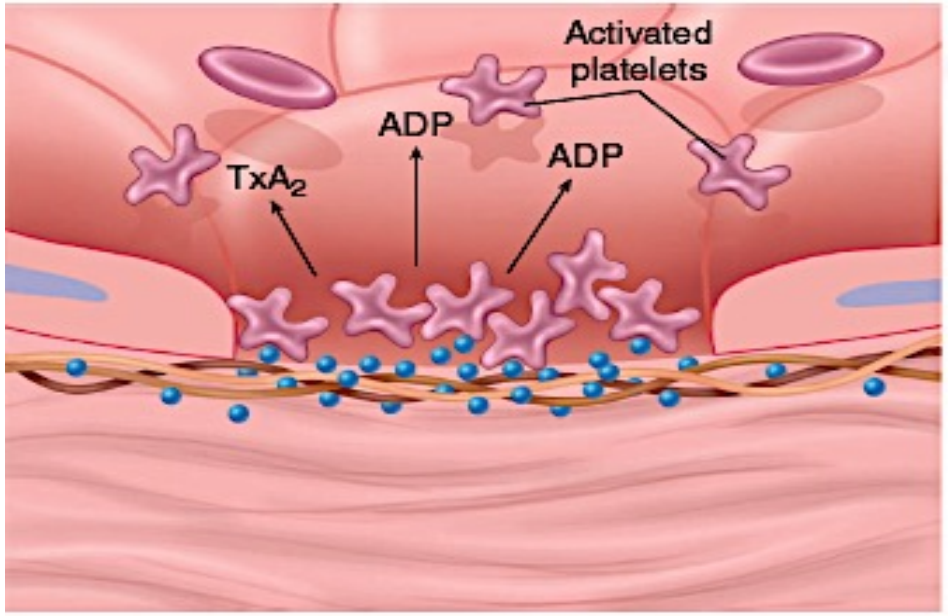
2)von Willebrand's factor (VWF) binds to both collagen and platelets → helps anchor the platelets

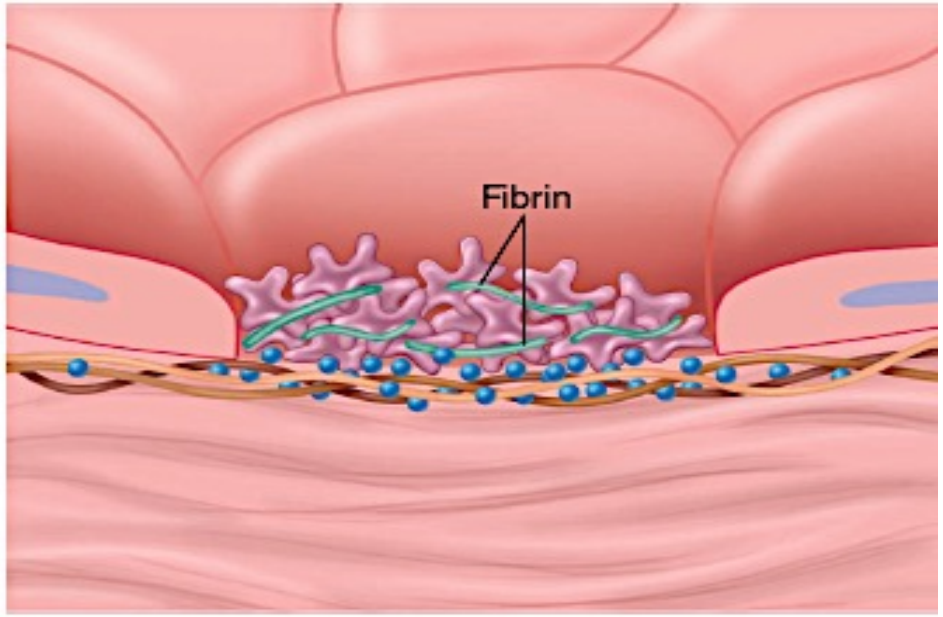
3)Platelets recruit more platelets and form a platelet plug by secreting:

a) ADP (sticky platelets)
b) Serotonin (vasoconstriction)
c) Thromboxane A (sticky platelets and vasoconstriction)

4) Activated platelets also activate plasma clotting factors

هذا هو السبب وراء وجود أنزيم CD39 بالوضع الطبيعي الي تم ذكره بالسلايد السابق ، وجود ADP بخلي الصفائح الدموية لاصقة و بتتجمع مع بعض لكن في الوضع الطبيعي احنا مش محتاجين تجمعهم هذا لذلك انزيم CD39 بحول ADP الى AMP + Pi .



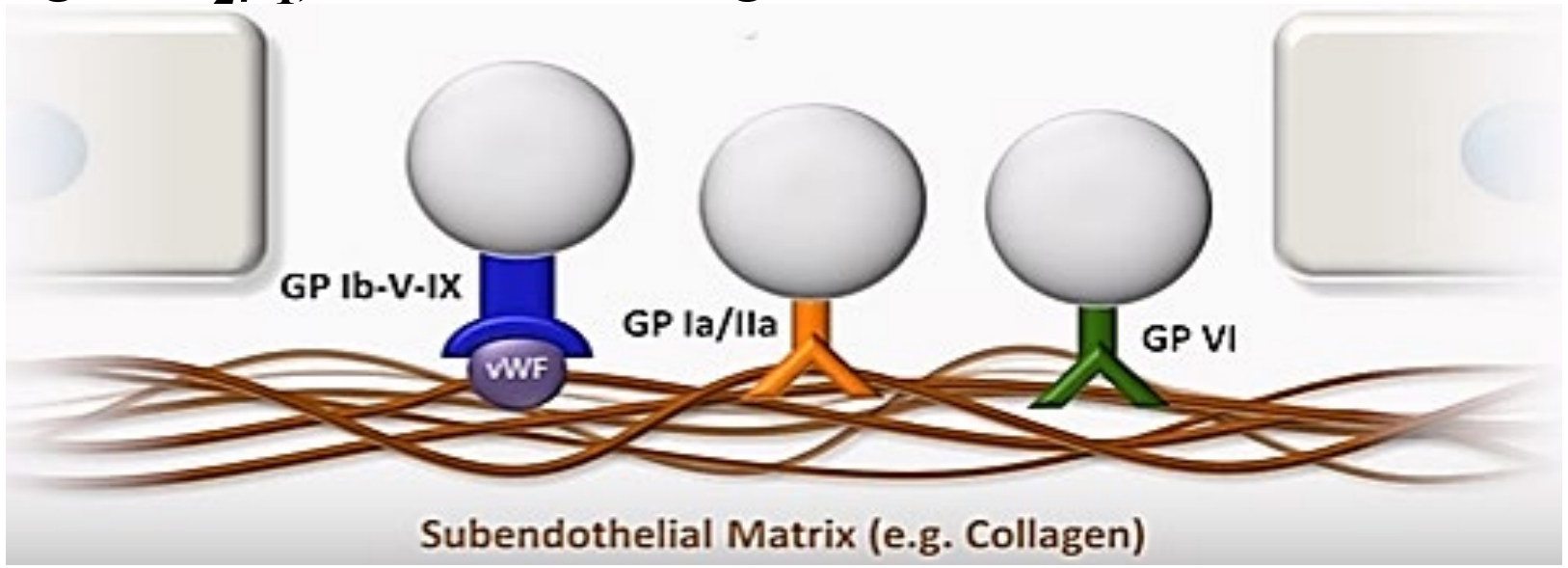


1. Formation of platelet plug

A. Platelets adhesion

- Triggered by exposure to Collagen
- The glycolprotein (GP) receptors expressed on platelets surface:

①GP Ib-V-IX complex form a bridging interaction with the exposed collagen that is mediated by vWF → The vWF recruits platelets to exposed collagen to prevent platelets from interacting with circulating vWF in undamaged areas
②GP Ia/IIa (also known as integrin $\alpha_2\beta_1$): Bind to collagen
③GP VI: Bind to collagen



B. Platelet Activation

Triggered by

① Collagen mediated platelet receptor binding, especially interaction with GP VI

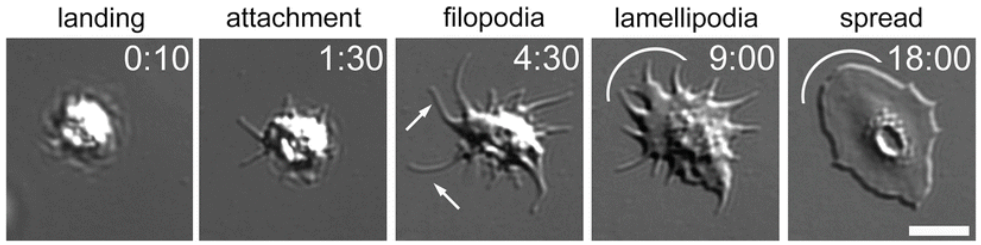
② Thrombin (produced from the initial response of the extrinsic pathway) bind to a class of G-protein-couple Receptor called Protease activating receptor (PAR1,PAR3, PAR4)

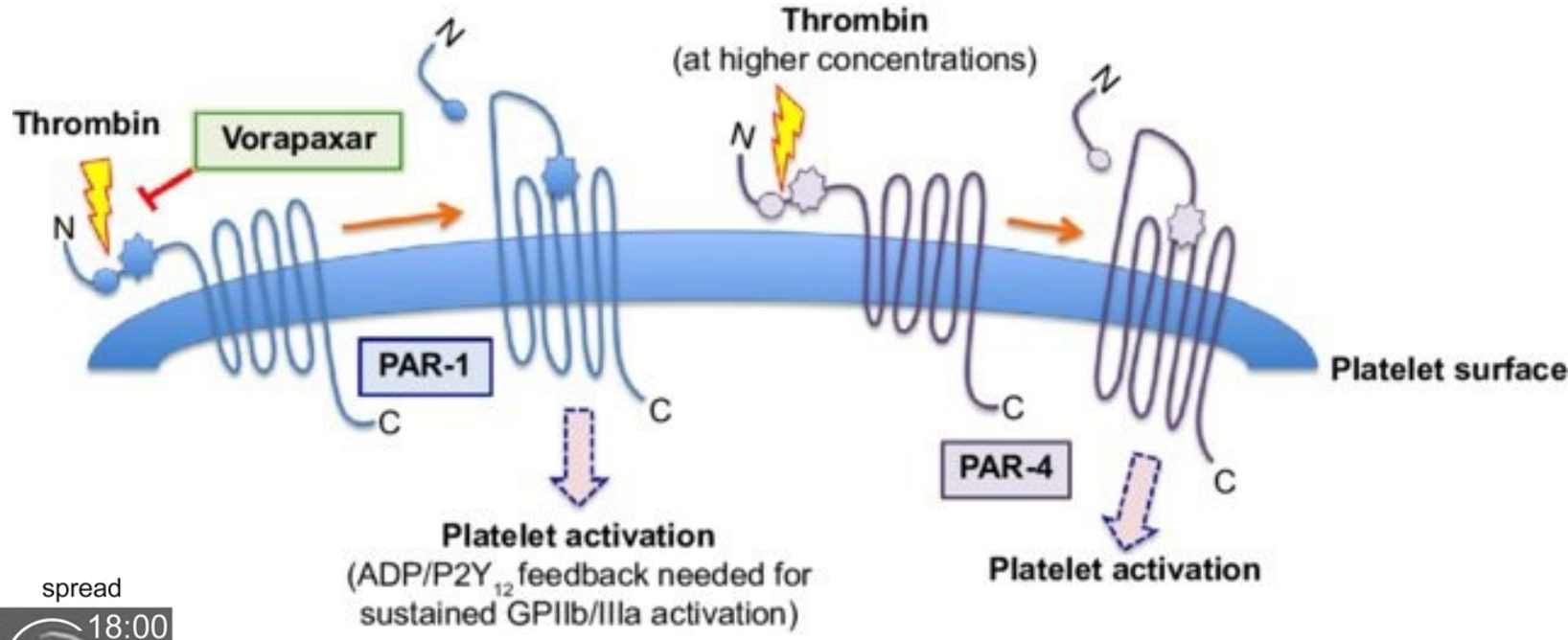
- The thrombin induced signaling leads to increase platelet activation and leukocytes adhesion
- Platelets are activated by second messenger signaling leads to:

① Activation of GP IIb/IIIa Granule secretion

③ Shape change →

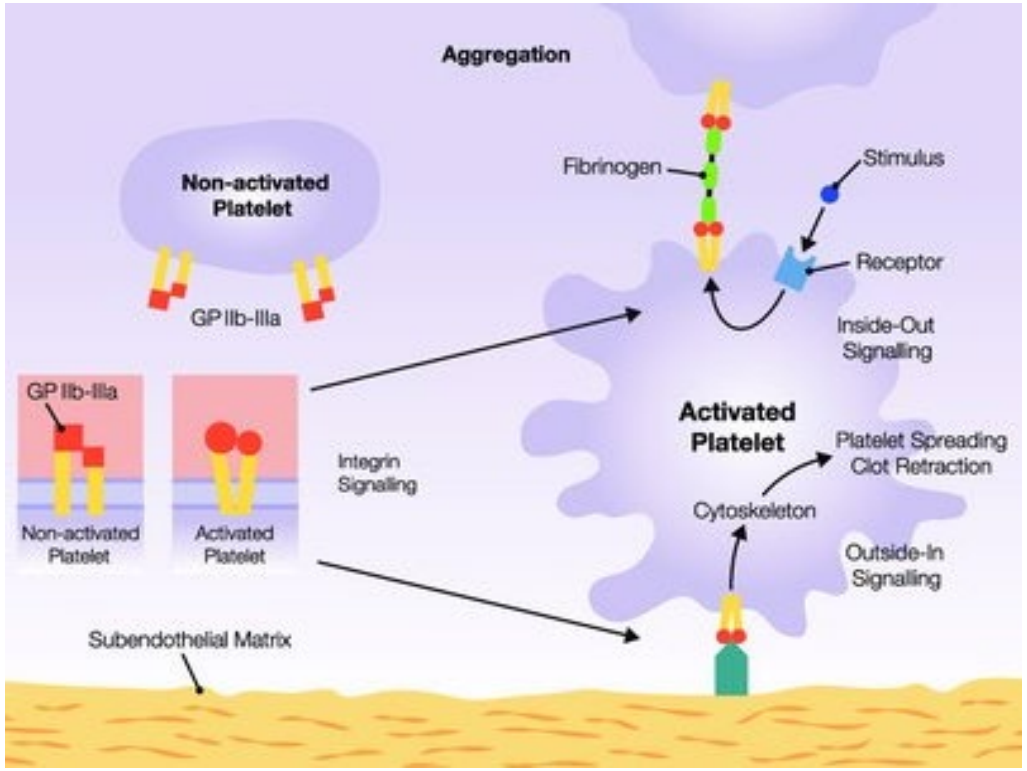
- They lose regular, discoid shape in response to increase intracellular Ca
- They become highly irregular with multiple filopodial extensions
- Their surface area increase
- They become sticky to neighboring platelets

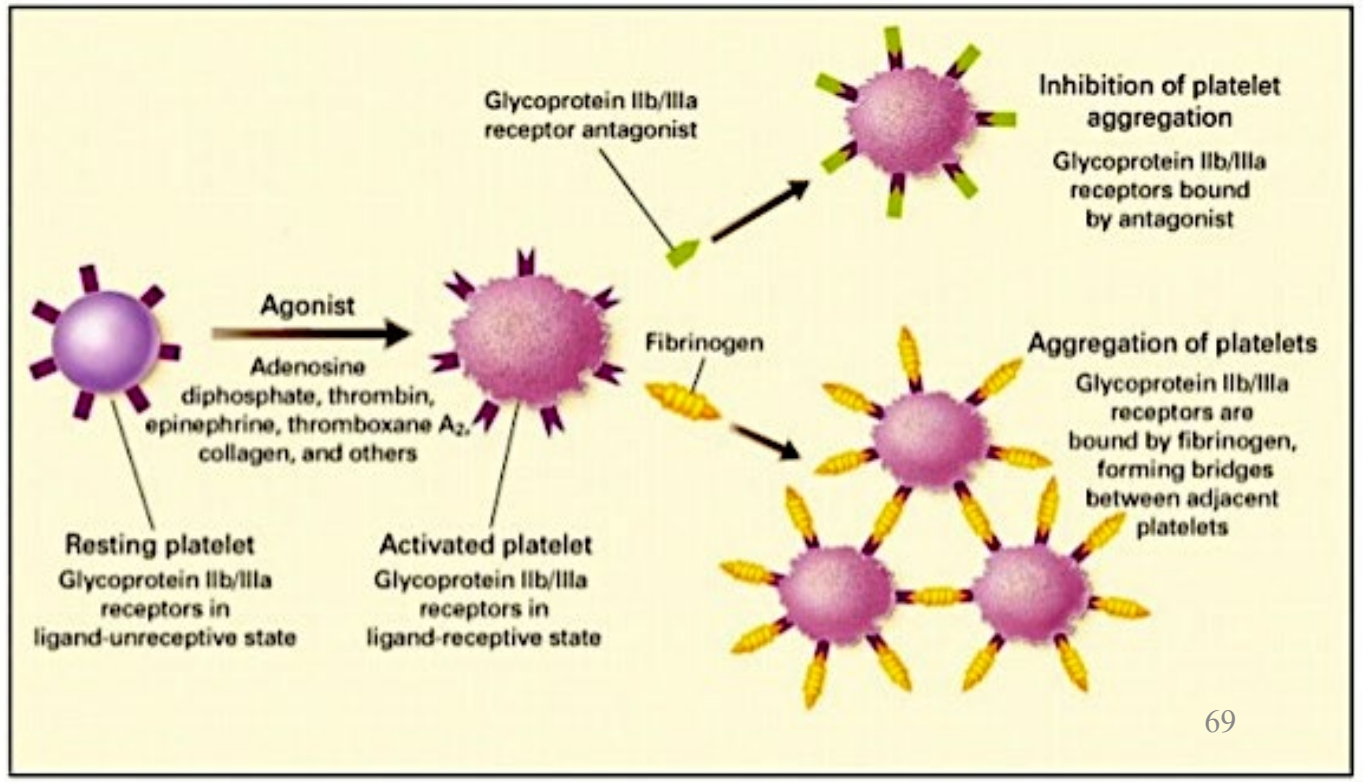




C. Platelet Aggregation

- It is mediated by a specific platelet receptor that binds to fibrinogen or fibrin.
- Only activated platelets have functional glycoproteins (GPs) IIb/IIIa (αIIbβ3 integrins) that bind to fibrin or fibrinogen (to prevent unwanted platelet aggregation).
- To promote platelet-to-platelet aggregation, fibrin and fibrinogen have multiple binding sites for the receptor→ one fibrin or fibrinogen molecule can bind to a GP IIb/IIIa on one platelet and at the same time bind to another GP IIb/IIIa on a second platelet.
- A single platelet contains many copies (about 50,000) of the GP IIb/IIIa receptor → each platelet can interact with many other platelets, thereby creating aggregated platelets →The final common pathway of platelet aggregation.





2. Initiation of coagulation (red clot formation)

Coagulation process is initiated by:

1. Trauma to the vascular wall and adjacent tissues
2. Blood trauma
3. Blood contact with damaged endothelial cells or with collagen and other tissue elements outside the blood vessel

Plasma Clotting Factors

- The clot is formed by the activation of the *blood-clotting factors proteins* →
- They are proteolytic enzymes in *inactive* forms →when they are activated → cause cascading reactions of the clotting process.
- Most of the clotting factors are designated by Roman numerals. To indicate the activated form of the factor, a small letter "a" is added after the Roman numeral, such as Factor VIIIa to indicate the activated state of Factor VIII
- **Serine proteases factors:** (#6) (Thrombin, F-VIIa, IXa, Xa, XIa and XIIa)
- **Cofactors:** (#4) (Tissue Factor, Ca ion, F-Va and VIIIa)

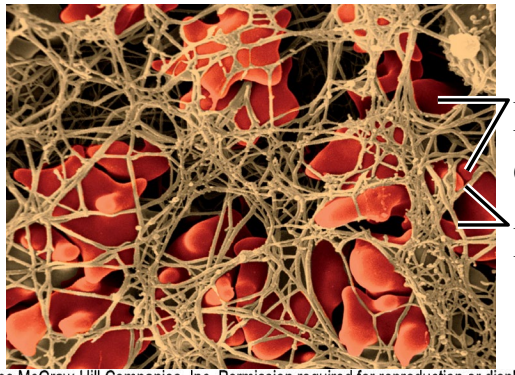


Table 13.4 | The Plasma Clotting Factors

Factor	Name	Function	Pathway
I	Fibrinogen	Converted to fibrin	Common
II	Prothrombin	Converted to thrombin (enzyme)	Common
III	Tissue thromboplastin	Cofactor	Extrinsic
IV	Calcium ions (Ca^{2+})	Cofactor	Intrinsic, extrinsic, and common
V	Proaccelerin	Cofactor	Common
VII*	Proconvertin	Enzyme	Extrinsic
VIII	Antihemophilic factor	Cofactor	Intrinsic
IX	Plasma thromboplastin component; Christmas factor	Enzyme	Intrinsic
X	Stuart-Prower factor	Enzyme	Common
XI	Plasma thromboplastin antecedent	Enzyme	Intrinsic
XII	Hageman factor	Enzyme	Intrinsic
XIII	Fibrin stabilizing factor	Enzyme	Common

*Factor VI is no longer referenced; it is now believed to be the same substance as activated factor V.

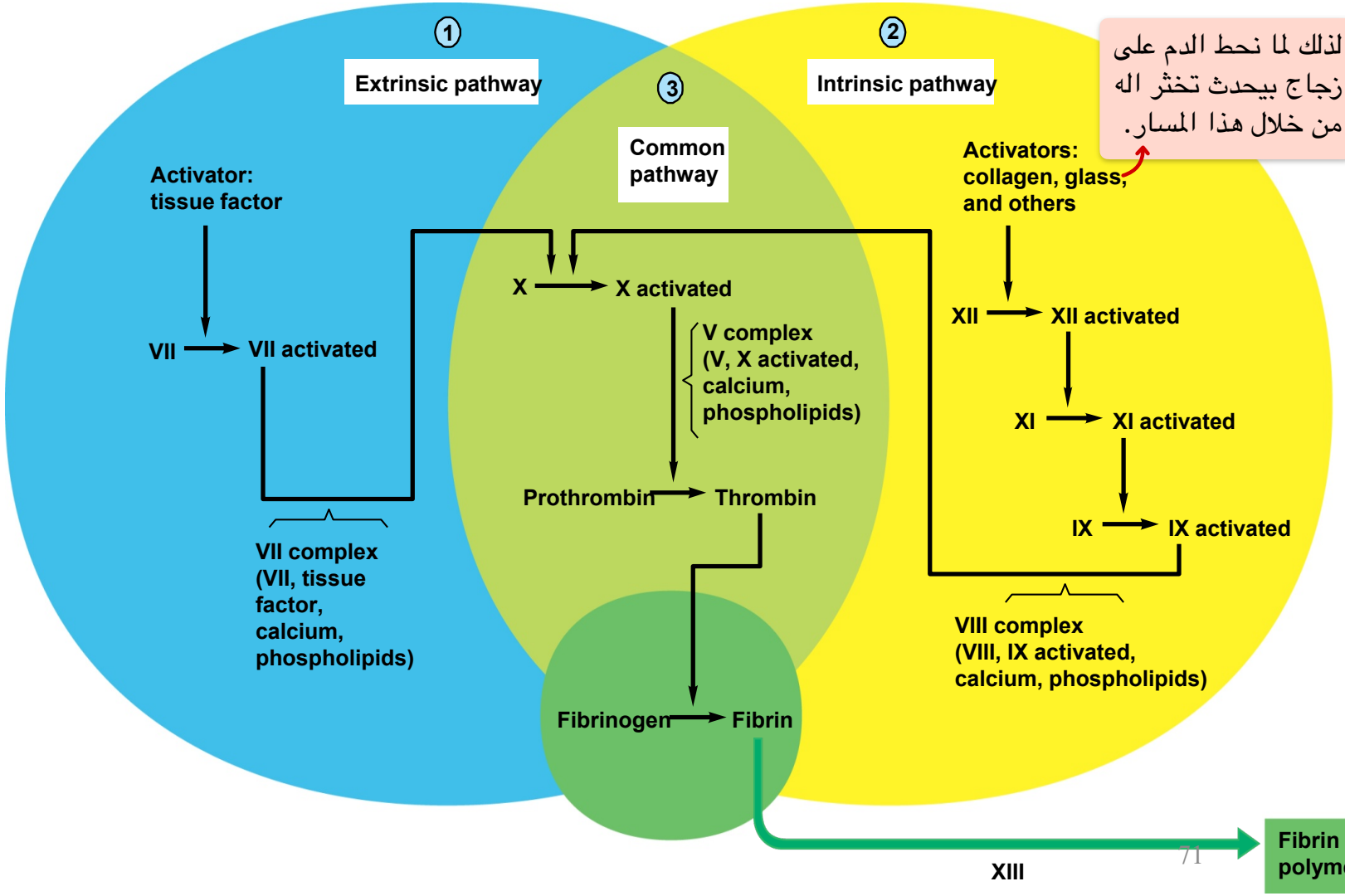
Clotting factors: Formation of Fibrin

Fibrinogen is converted to fibrin via one of two pathways:

بعد ما تم تكوين platelet plug بدنا نحط Fibrin عشان يثبت platelet plug و ما نفقدها بسبب جريان الدم ، ويتم ذلك عبر بطريقتين.

1. Extrinsic pathway:

Damaged tissue release a chemical that initiates the formation of fibrin → Initiated by tissue factor thromboplastin (factor III) secreted by the subendothelial cells and leukocyte after external damage. →This is a more direct pathway (shortcut).



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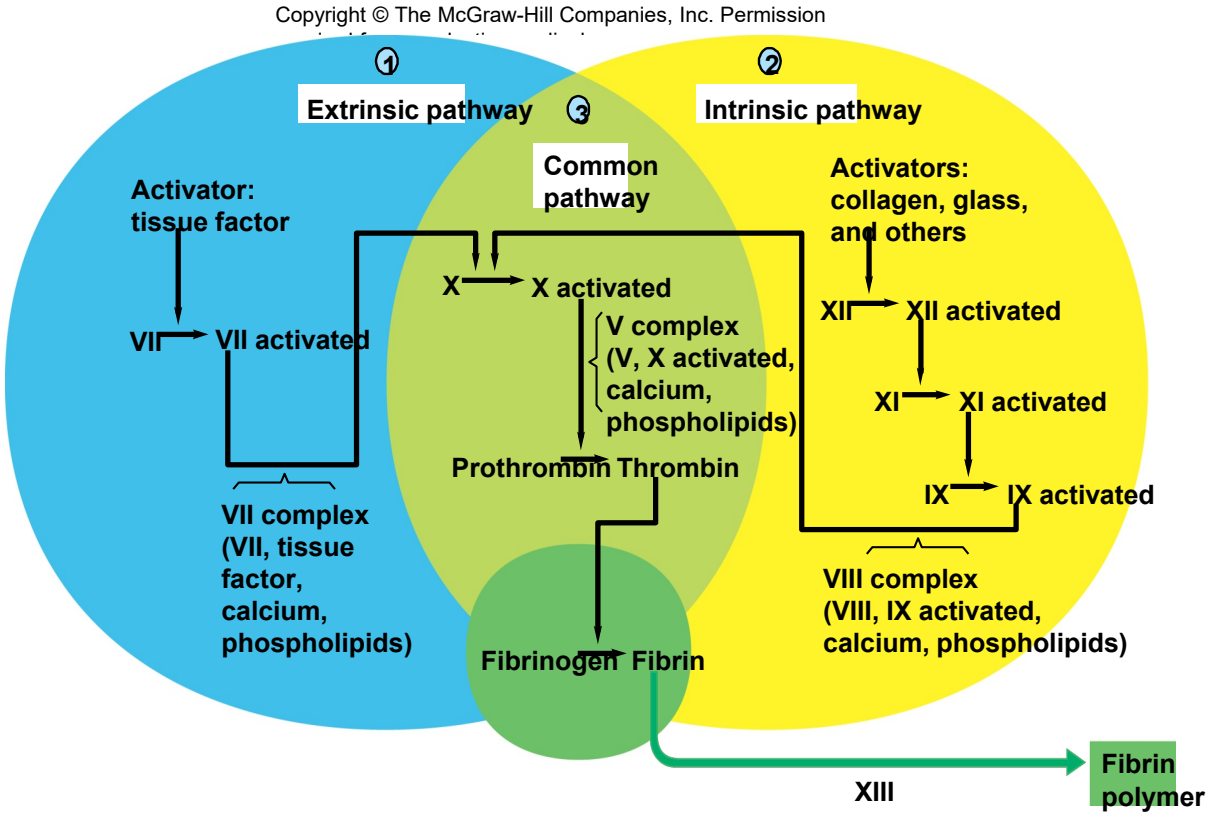
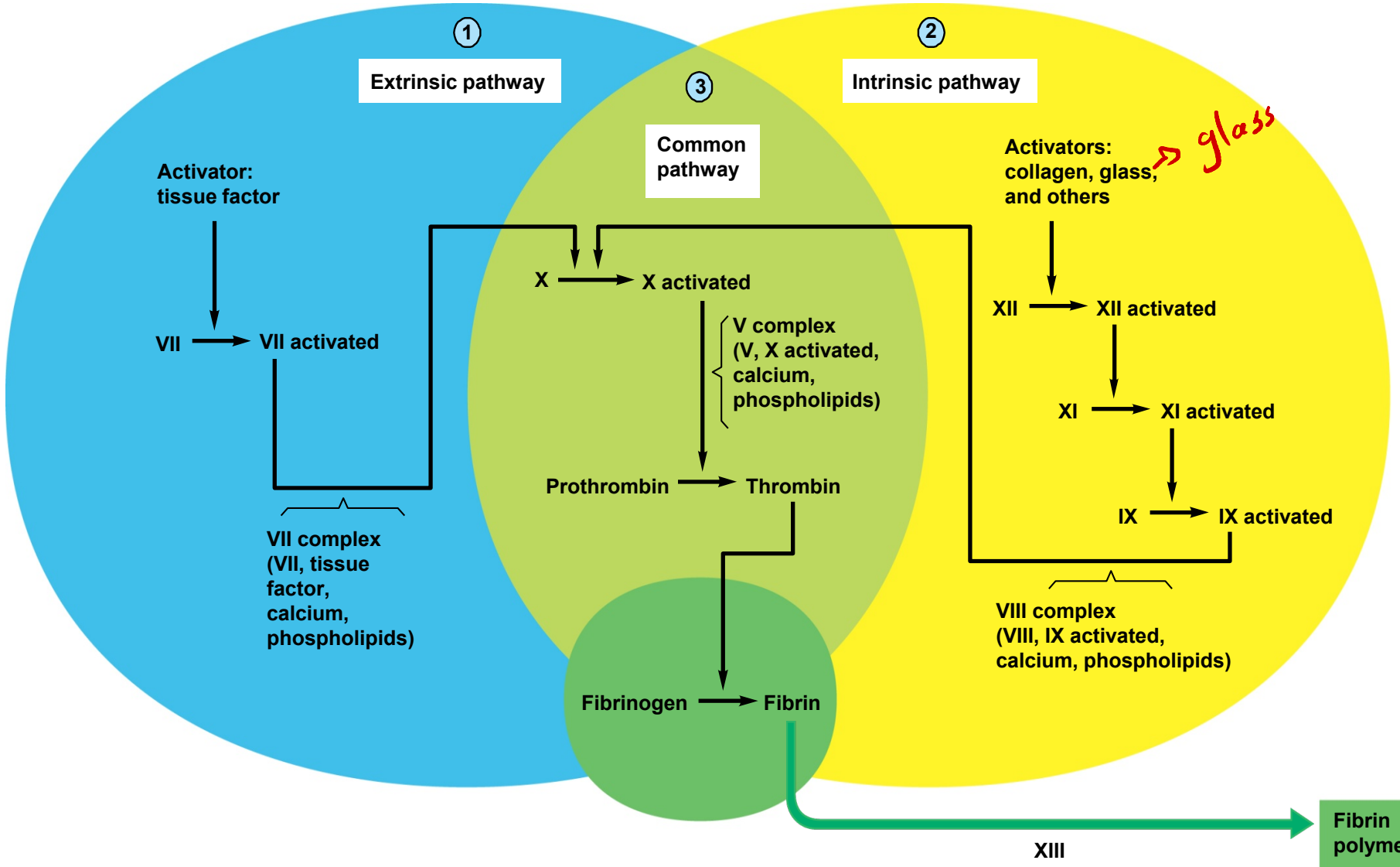
Continue….

1. Begins with a traumatized vascular wall or traumatized extravascular tissues that come in contact with the blood
2. Tissue factor initiates the extrinsic pathway→ result in
3. Release of tissue factor (thromboplastin):
4. Activation of Factor X by Factor VII and tissue factor: Factor VII in the presence of Ca ions, acts enzymatically on Factor X to form *activated Factor X* (Xa).
5. Factor V interact with Xa to form prothrombin activator complex in the presence of Ca and tissue phospholipids. → prothrombin is split to form thrombin.



2. Intrinsic pathway:

Blood left in test tube will clot without the addition of any chemicals → Activated by exposure to collagen→ Factor XII activates a cascade of other blood factors



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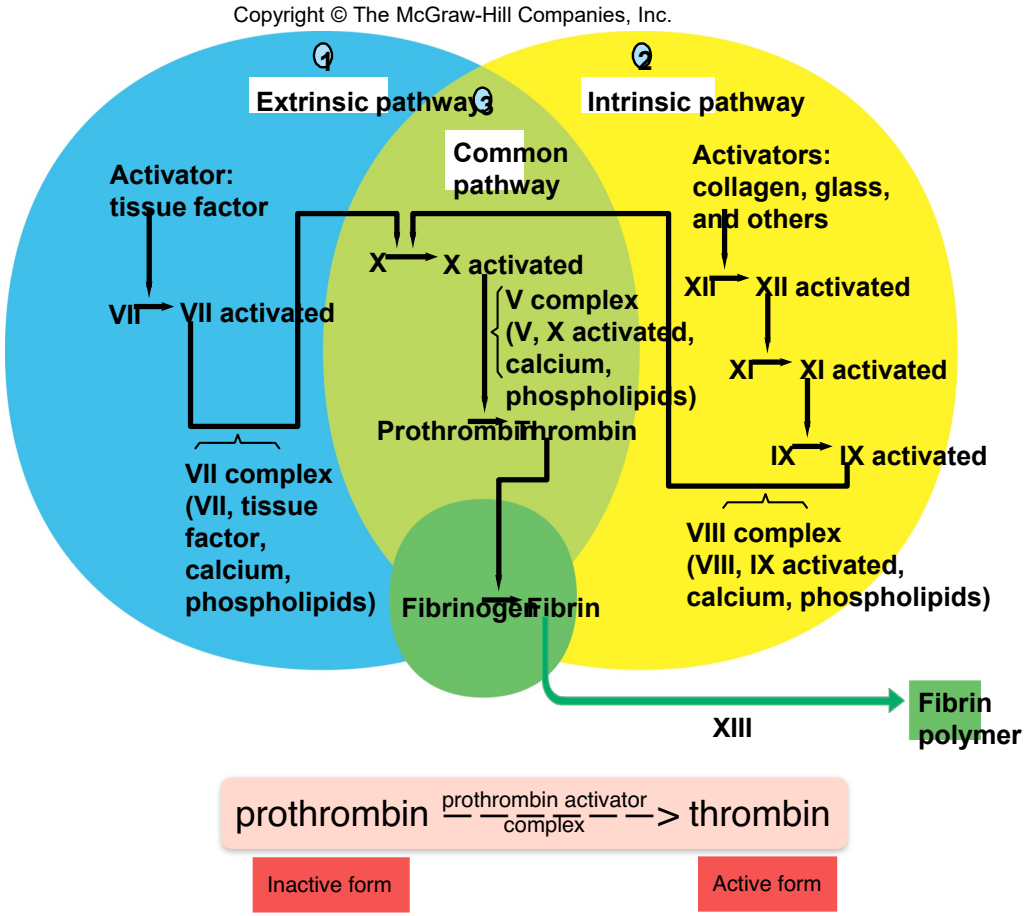
1. Begins with trauma to the blood or exposure of the blood to collagen from a traumatized blood vessel wall
2. The contact of Factor XII and platelets with collagen in the vascular wall initiates the intrinsic pathway → cause
3. **The activation of Factor XII** → Factor XIIa
4. **The release of Platelet phospholipids** that contain the lipoprotein called *platelet factor 3 (*plays a role in subsequent clotting reactions)
5. **Activation of Factor XI:** The activated Factor XII acts enzymatically to activate Factor XI. This reaction also requires *high-molecular-weight kininogen* and is accelerated by prekallikrein.
6. **Activation of Factor IX by activated Factor XI.** The activated Factor XI then acts to activate Factor IX
7. **Activation of Factor X**. The activated Factor IX with activated Factor VIII and with the platelet phospholipids and Factor III from the traumatized platelets, activates Factor X.
8. **Factor X action to form prothrombin activator**. Activated Factor X combines with Factor V and platelet or tissue phospholipids to form prothrombin activator complex→ which in turn initiates the cleavage of prothrombin to form thrombin
9. **The prothrombin activator**, in the presence of Ca causes the conversion of prothrombin to thrombin.
10. **The thrombin converts fibrinogen** (soluble) into fibrin fibers (insoluble) that trap platelets, blood cells, and plasma to form the clot.



3. Common pathway:

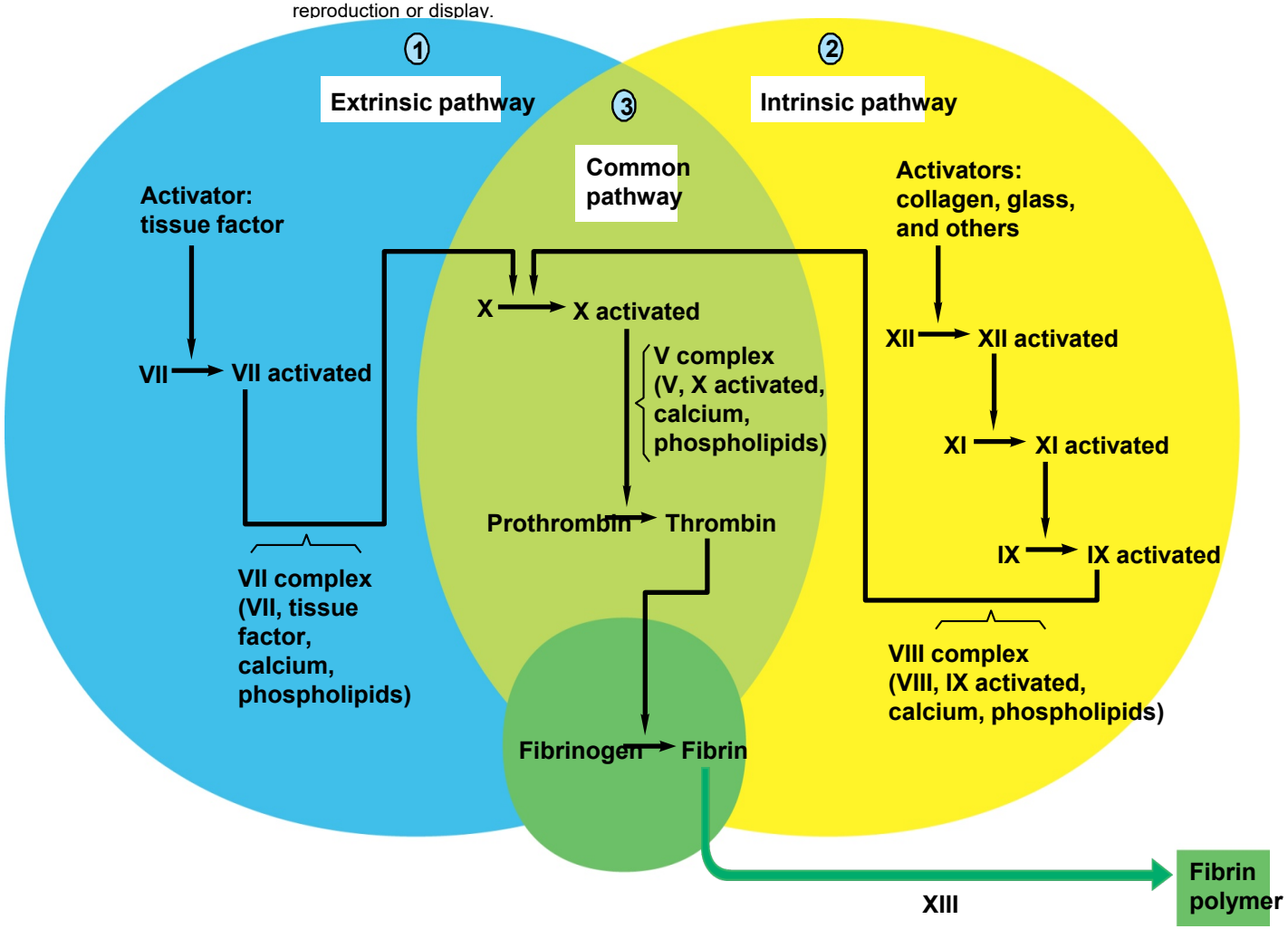
- Both intrinsic and extrinsic pathways lead to activate

Factor X → activated factor Xa

→Prothrombin → Thrombin

→Fibrinogen → Fibrin

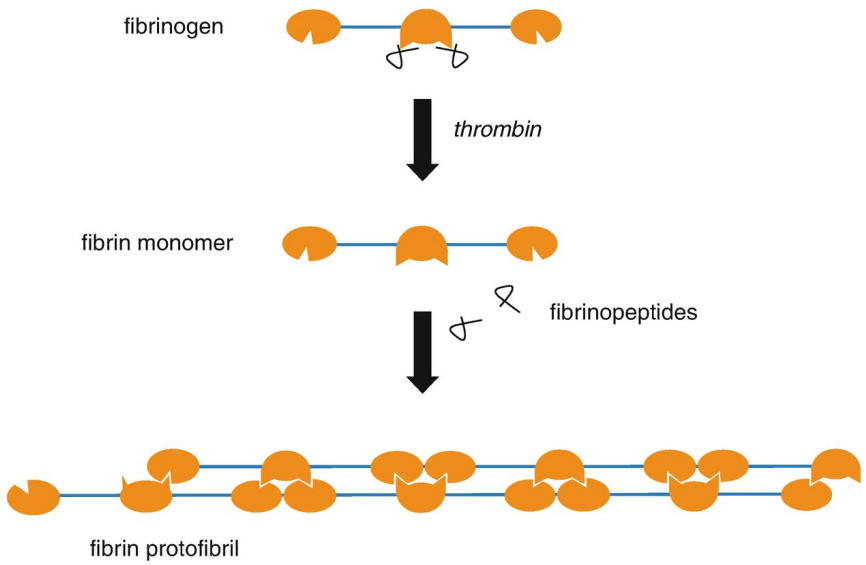
- This is mediated by **Ca** and **phospholipids** (from the platelets)

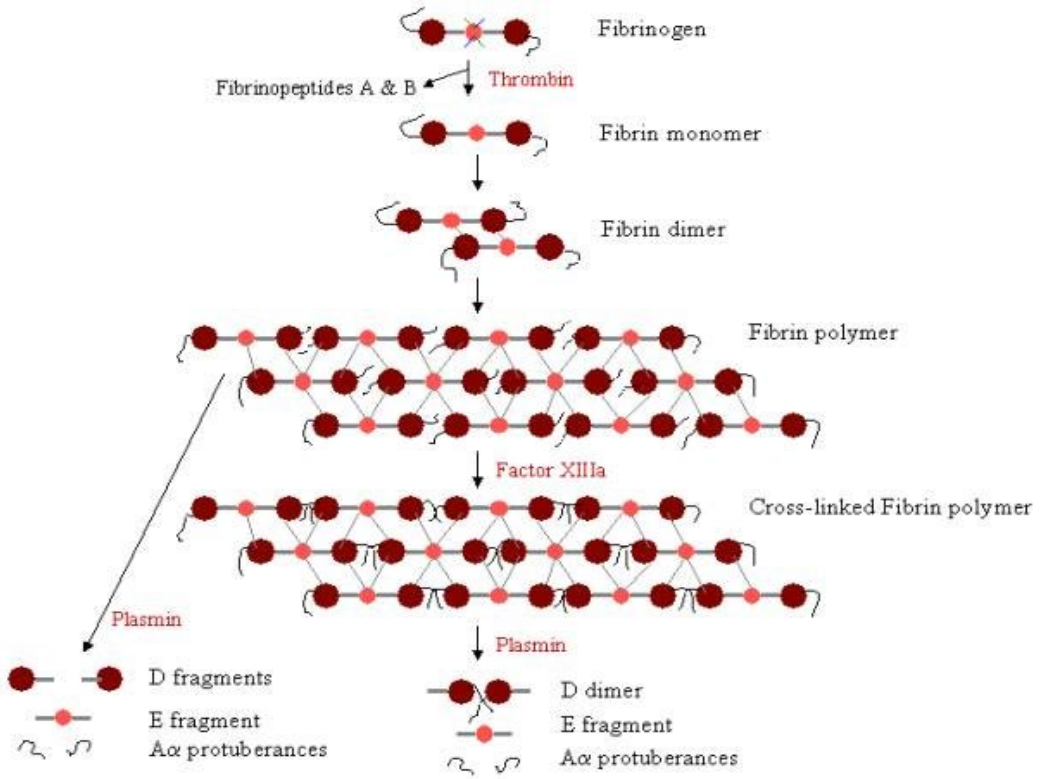


4. Formation of Fibrin polymer

1. The formation of fibrin involves the release of two pairs of polypeptides from each fibrinogen molecule.
2. The remaining portion, **fibrin monomer,** then polymerizes with other monomer molecules to form **fibrin.**
3. The fibrin (loose mesh of interlacing strands) is converted by the formation of covalent cross-linkages to a dense, tight aggregate (stabilization) → This reaction is catalyzed by →
4. **Activated factor XIII** (fibrin-stabilizing factor) which causes more and more cross-linking bonds between adjacent fibrin fibers
5. This step requires Ca

- Deficiency of XIII affects clot stability and increases bleeding tendency.
- The rate-limiting factor in causing blood coagulation is the formation of prothrombin activator that convert prothrombin to thrombin
- **Plasmin** digests fibrin → Dissolution of clots
- When clots are formed within blood vessels, activation of plasminogen to plasmin may lead to their removal.
- **Vitamin K** is needed by the liver to make several of the needed clotting factors

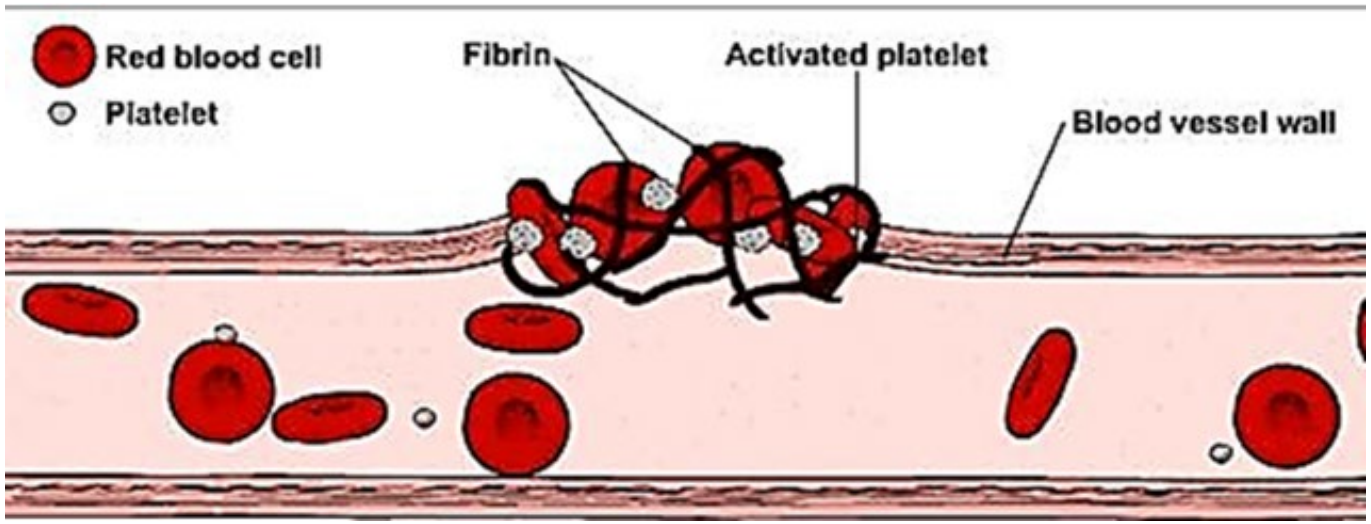




Platelets role in the coagulation process

- The platelets contribute directly to clot contraction by activating platelet

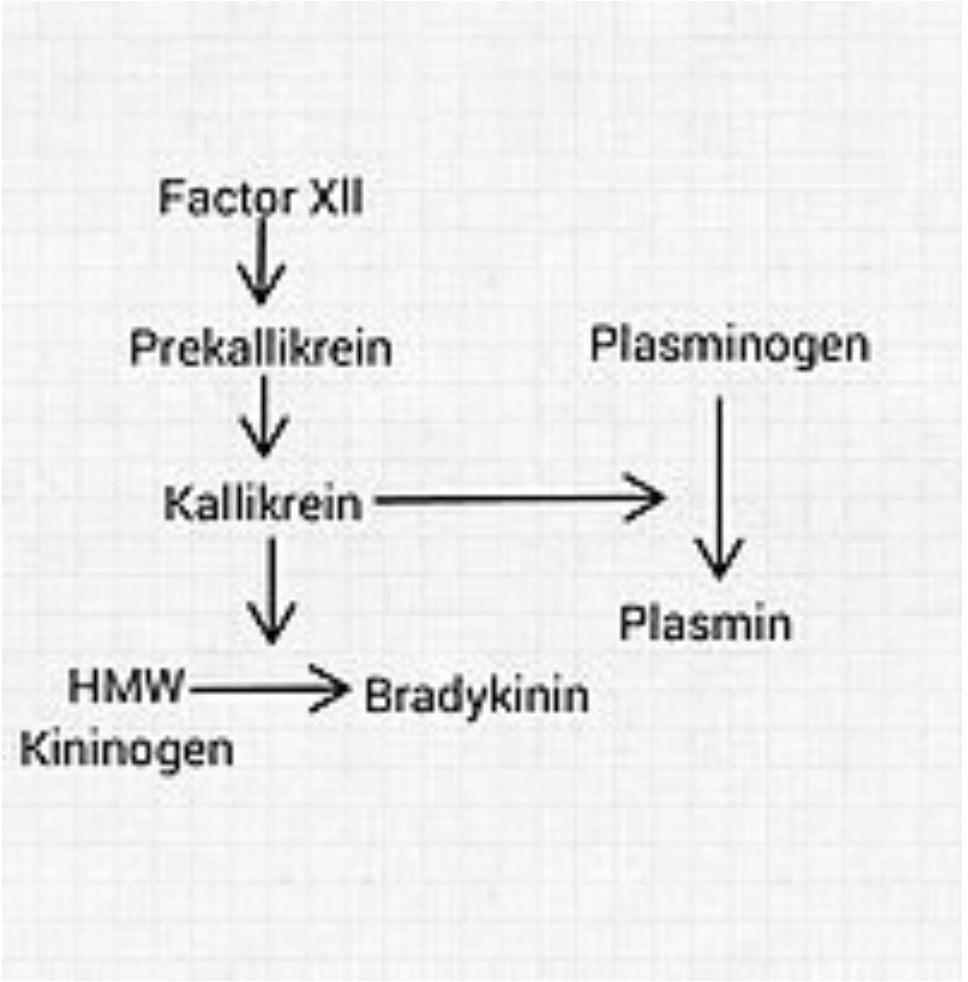
1. Thrombosthenin (A contractile protein from thrombocytes)
2. Actin, and myosin (contractile proteins in the platelets) →cause strong contraction of the platelet spicules attached to the fibrin→ compress the fibrin meshwork into a smaller mass.
3. The contraction is activated and accelerated by thrombin and Ca
4. As the clot retracts, the edges of the broken blood vessel are pulled together, thus contributing further to hemostasis



The role of other proteins in the coagulation process

- **Prekallikrein (PK):** protein that complexes with **kininogen**. PK is the precursor of plasma **kallikrein**, which is a serine protease that activates kinins. PK is cleaved to produce kallikrein by activated Factor XII
- **Kininogens:** is a circulating high molecular weight plasma protein which participates in the initiation of blood coagulation, and in the generation of the vasodilator bradykinin via the kallikrein-kinin system.
- **Bradykinin** is the end-product of the contact activation system. This enzymatic cascade circulates in the plasma and consists of factor XII, plasma prekallikrein and high molecular weight kininogen → cause vasodialation

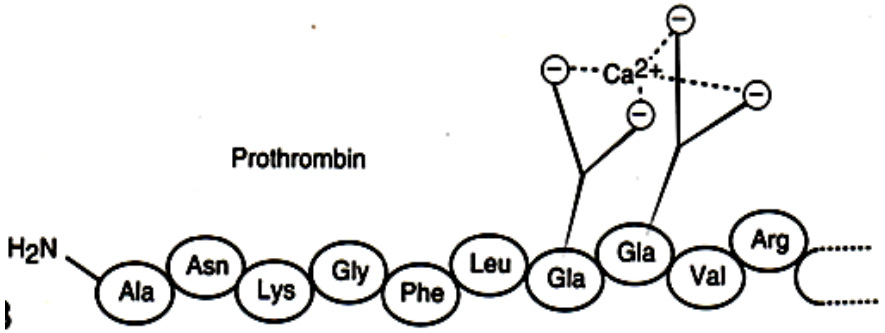
→*This system is linked to the intrinsic coagulation system via factor XI*

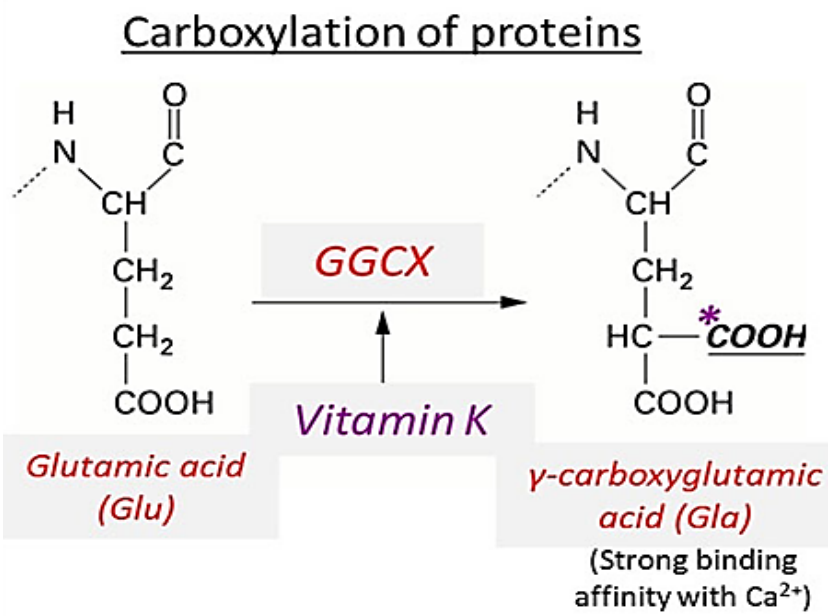


الدكتورة قالت اهم اشي و الي بهمنا في هذا السلايد نكون عارفين اخر نقطتين هم انه بعمل vasodialation and intrinsic coagulation .

Vitamin K role in the coagulation process

- It is involved in the formation of gamma-carboxy-glutamate residues which occurs during synthesis of clotting factors in the liver.
- The Carboxy-glutamic acid (carboxy group is added to glutamic acid) present in the clotting factors introduces an affinity for Ca ions → required for binding to platelet membrane phosphatidylserine (PS)
- In the blood coagulation cascade, Vitamin K is required to introduce gamma-carboxylation of clotting factors II, VII, IX, X.
- Non-carboxylated factors are "**inactive**" because they cannot bind Ca or to PS, therefore, cannot participate in the formation of the essential coagulation complexes that are required for fibrin formation.





Role of Calcium Ions in the Intrinsic and Extrinsic Pathways

- Ca ions are required for promotion or acceleration of all the blood-clotting reactions.→ Therefore, in the absence of Ca ions, blood clotting by either pathway is inhibited
- Clotting can be prevented with certain drugs:

  1) Calcium chelators (sodium citrate, EDTA)

  2) Heparin: blocks thrombin (The heparin molecule is negatively charged it has little or no anticoagulant properties, but when it combines with antithrombin III, the effectiveness of antithrombin III for removing thrombin increases by a hundredfold to a thousand fold, and thus it acts as an anticoagulant)

  3) Coumarin: inhibits vitamin K

Summary of the coagulation process

tenase:activation factor X

- **The activating complexes** are together called **tenase (**the complex activates its substrate (inactive factor X) by cleaving it)
- Extrinsic tenase complex is made up of tissue factor, factor VII, and Ca^{2+} as an activating ion.
- Intrinsic tenase complex contains the active factor IX (IXa), its cofactor factor VIII (VIIIa), the substrate (factor X)

1. **Initial Phase:** formation of (TF-FVIIa): extrinsic tenase complex

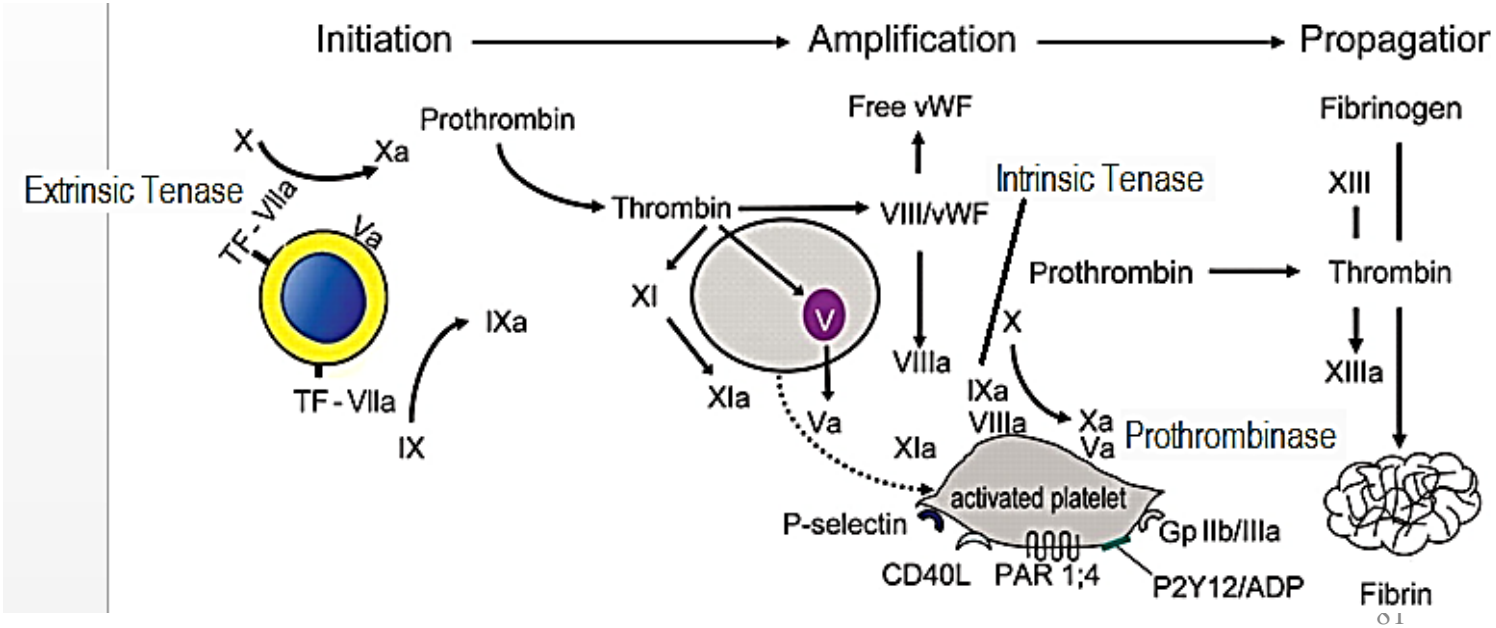
TF: tissue factor

2. **Amplification phase**: activating platelets as well as FV, FVIII, and FXI

F: factor

3. **Propagation phase:** formation of (IXa-VIIa): intrinsic tenase complex and (Xa-Va): prothrombinase →In the propagation phase, a burst of thrombin is generated, which is sufficient for the clotting of fibrinogen and formation of a fibrin meshwork. A thrombus is formed.

	Complex	Proteinase	Cofactor	Substrate
extrinsic tenase	VIIa,TF,Ca, Phospholipid	VIIa	Tissue factor	X
intrinsic tenase	IXa,VIIIA,Ca, Phospholipid	IXa	VIIIa	X
prothrombinase	Xa,Va,Ca, Phospholipid	Xa	Va	Prothrombin



Factors interfere with the clotting mechanism

A. Anti-clotting mechanism

- *The Endothelial Surface Factors that prevent clotting in the normal vascular system are:*

(1) The **smoothness** of the endothelial cell surface, which prevents contact activation of the intrinsic clotting system.

(2) **The Glycocalyx layer** (a mucopolysaccharide adsorbed to the surfaces of the endothelial cells) on the endothelium → repels clotting factors and platelets, thereby preventing activation of clotting

(3) Endothelium secret **Nitric oxide (NO)** which causes vasodilation and inhibits platelet aggregation

(4) Endothelium secret **prostaglandin** which causes vasodilation and inhibits platelet aggregation

(5) **Anti-thrombin III** (antithrombin-heparin cofactor)→ The intact endothelium adjacent to the damaged tissue actively secretes several anti-thrombotic substances including *heparan sulfate, and thrombomodulin* →The thrombin that does not adsorb to the fibrin fibers combines with antithrombin III, which further blocks the effect of the thrombin on the fibrinogen →inactivates the thrombin itself during the next 12 to 20 minutes

→Antithrombin III inactivates Factors Xa, IXa, XIa, XIIa, thrombin (IIa),VIIa, kallikrein and plasmin

(6)**Thrombomodulin**: Most of the endothelial cells produce **thrombomodulin (**a thrombin-binding protein) on their surfaces. When thrombin binds to thrombomodulin it becomes an anticoagulant forming thrombomodulin-thrombin complex, which activates protein C (APC).

→APC inactivates factors V and VIII and increase the formation of plasmin.

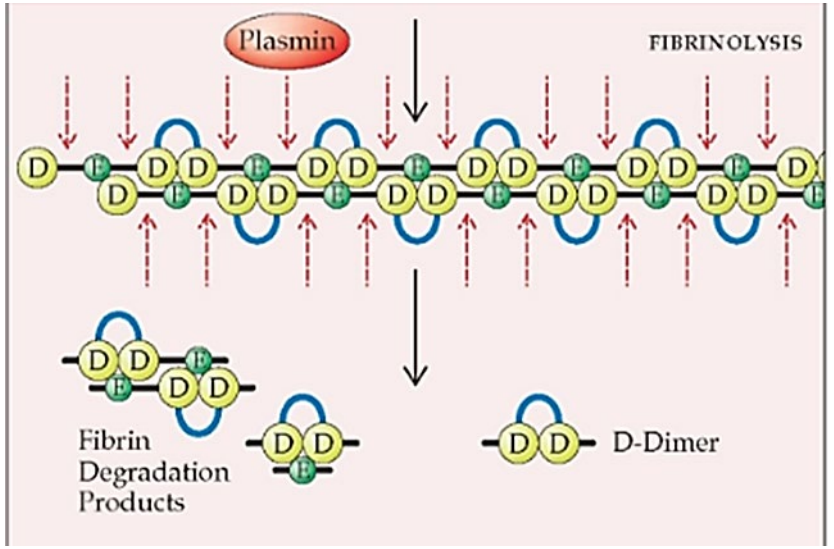
B. Tissue factor pathway inhibitor (TFPI)

- TFPI inhibits the extrinsic coagulation pathway

→ it inhibits Factor Xa and Factor VIIa-tissue factor complex

C. Fibrinolytic system

- Plasminogen which is formed from in the liver → when activated, becomes plasmin
- Plasmin is a proteolytic enzyme that resembles trypsin (digestive enzyme of pancreatic secretion).
- Plasmin degrades the fibrin mesh into soluble end products known as fibrin split products
- plasmin cause lysis of a clot by destroying many of the clotting factors→ thereby causing hypo-coagulability of the blood.



Bleeding disorders

- A bleeding disorder is a condition that affects the normal blood clotting process
- In people with bleeding disorders: the clotting factors or platelets don't work the way they should or are in short supply.
- When the blood doesn't clot, excessive bleeding can occur in the muscles, joints, or other parts of the body.
- The majority of bleeding disorders are inherited

Bleeding may caused by:

1. A low RBC count
2. Vit K deficiency
3. As a side effects from certain medications
4. Common bleeding disorders

a) Hemophilia A and B conditions that occur when there are **low levels** of clotting factors in blood → It causes heavy or unusual bleeding into the joints. (rare)

b) Factor II, V, VII, X, or XII deficiencies

c) Von Willebrand's disease: the most common inherited bleeding disorder. It develops when the blood lacks von Willebrand factor, which helps the blood to clot.

Bleeding time, Clotting time and Prothrombin time

- **Bleeding times**: the time needed after a cut in the tissue to stop bleeding (normally 1-3 min)

- **Clotting time**: the time needed for the blood to make a clot (normally 5-8 min)
- **Prothrombin time (PT):** measures the clotting tendency of the blood, it is an assay for evaluating the extrinsic pathway and common pathway of coagulation.
- PT measures the blood-thinning medication warfarin (anticoagulant) dosage, liver damage and diseases (used to screen people waiting for liver transplants), Vit K status, Coagulation factors (I, II, V, VII, X)

Thrombin provides positive feedback

1. The intrinsic pathway by converting factor VIII to factor VIIIa and factor XI to factor XIa
2. The common pathway by converting factor V to factor Va;
3. Platelets by binding to thrombin receptors to amplify platelet activation -hard clot production by converting factor XIII to factor XIIIa.
4. Factor VII to factor VIIa
5. Factor XI to factor XIa

فيديو مراجعة قصير مدته ٥ دقائق استخدمته الدكتورة في نهاية المحاضرة.

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