بسم الله الرحمـٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

HLS BioChemistry | MID L4+5

Blood Coagulation



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بسم الله الرحمن الرحيم،

نرجو منكم الدعاء لزميلنا في الدفعة
عون العدوان، الذي وافته المنية.

اللهم ارحمه واغفر له، وأسكنه فسيح جناتك، وأنر قبره وآنس وحشته، واجعل قبره روضةً من رياض الجنة.

اللهم اجبر قلوب أهله وذويه، وارزقهم الصبر والسلوان، وألهمهم جميل الصبر وحسن العزاء.

رحمه الله رحمةً واسعة وأسكنه الفردوس الأعلى من الجنة...

Blood coagulation

Prof. Mamoun Ahram
Minor edit by : motamed qadan
Hematopoietic-lymphatic system

Resources

- This lecture
- Harper's Medical Biochemistry, 31st edition, Chapter 55
- Mark's Basic Medical Biochemistry, 7th edition, Chapter 43
- Ninja Nerd Official youtube

Hemostasis

- Hemostasis refers to the stopping of bleeding (hemo = blood, stasis = stopping).

- It must be distinguished from homeostasis, the physiological chemical balance of the body.

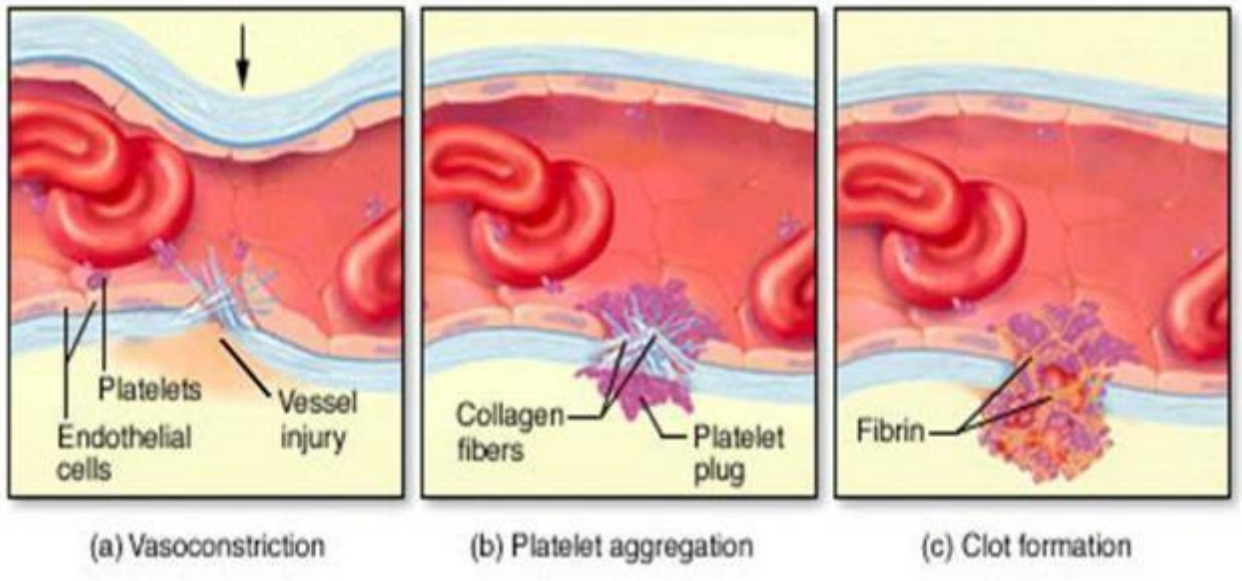
- The process occurs in four sequential steps :
  1. Vasoconstriction brings endothelial cells closer to reduce blood leakage.
  2. Primary hemostasis forms a temporary “soft clot.”
  3. Secondary hemostasis stitches the soft clot together, binding it tightly to create a “hard clot.”
  4. Dissolution.

Steps of hemostasis and thrombosis

First step to take place immediately after injury is

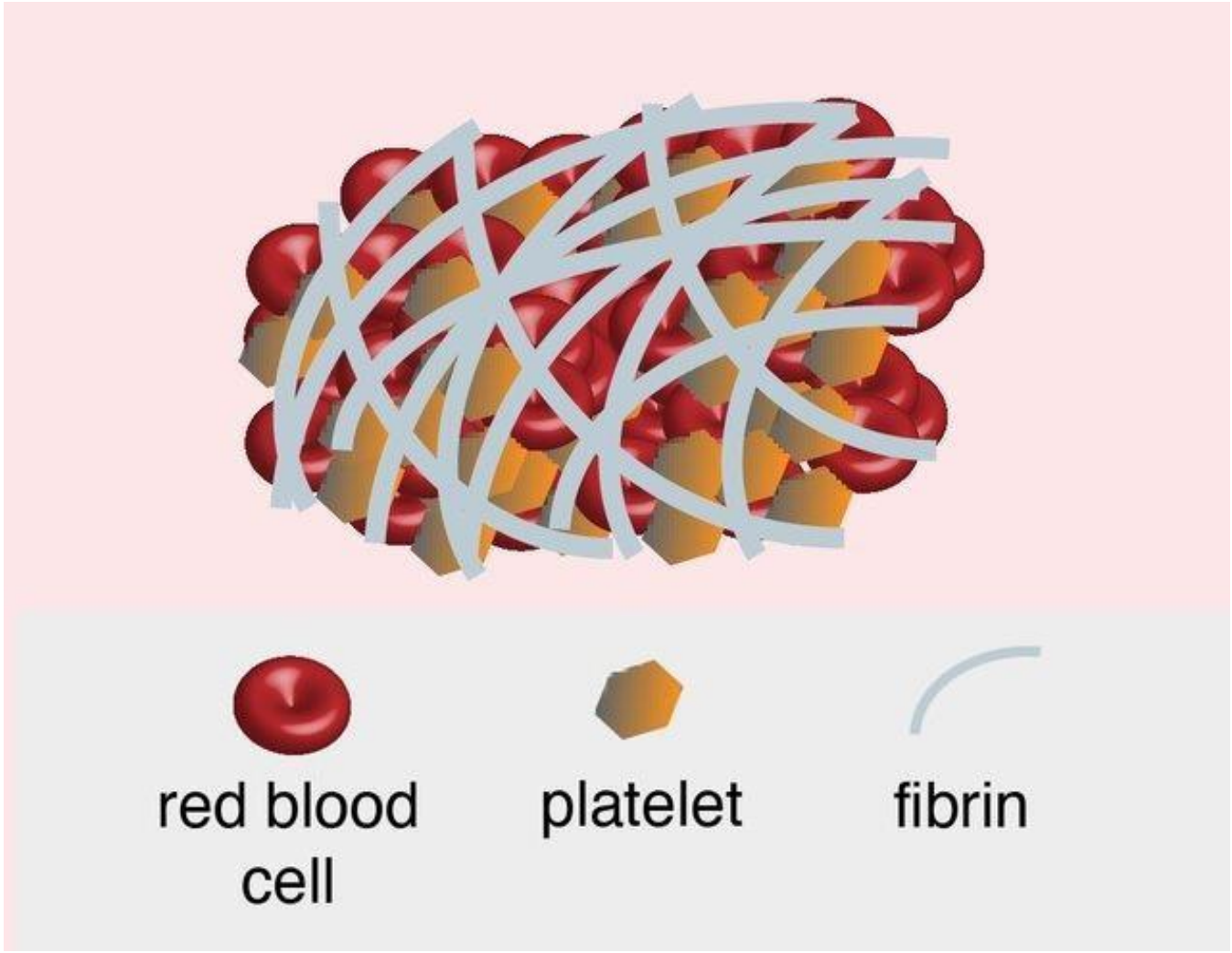
- Vascular constriction limiting blood flow to the area of injury by slowing down blood flow and pulling the edges together.
- Activation then aggregation of platelets at the site of injury, forming a loose platelet plug
- Formation of a fibrin mesh to entrap the plug, this newly formed fibrin mesh is actually firm.
- Dissolution of the clot in order for normal blood flow to resume following tissue repair

Homeostasis: how your body systems regulate and maintain themselves.
Hemostasis: prevention of blood loss.



Composition of a clot

As we can see 3 components make up a clot but we'll only be discussing 2 of them (platelet, fibrin).

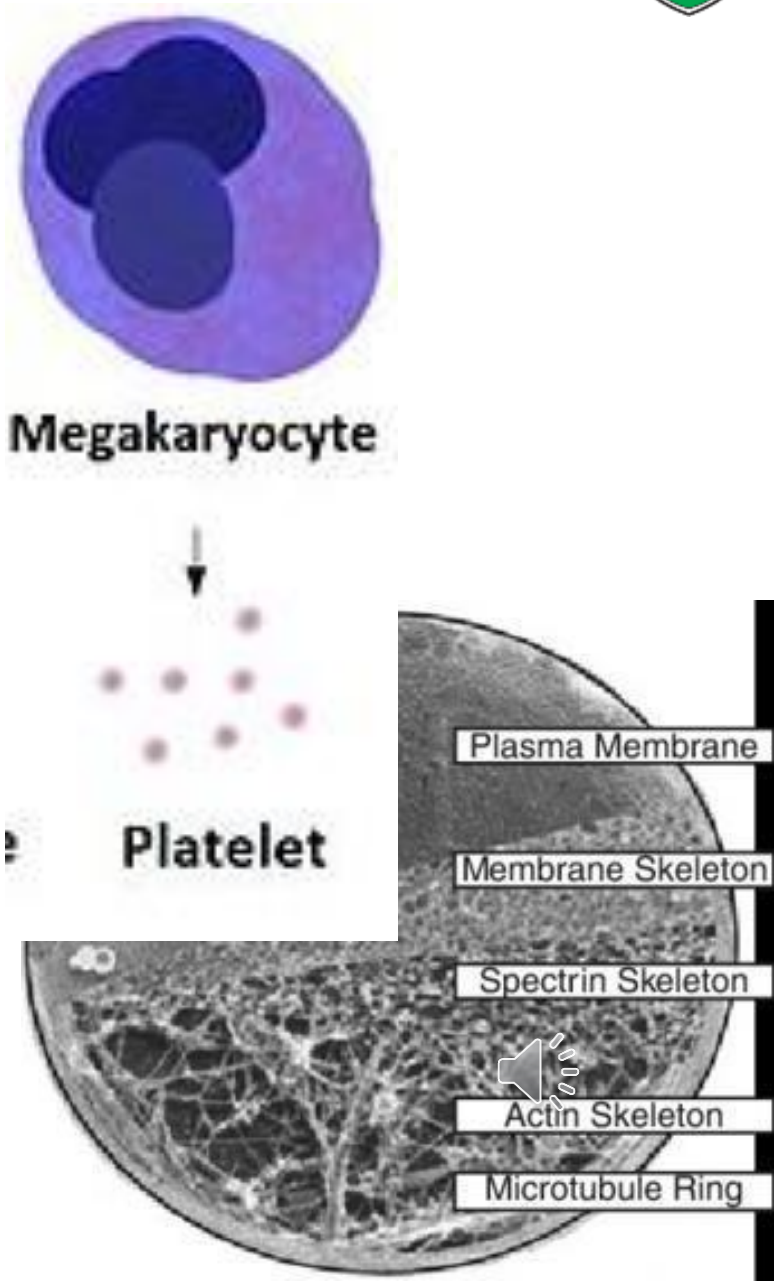


Platelets are a major player

- **Platelets have receptors that bind to the injured endothelium** (for example, **GP Ib binding to von Willebrand factor**), receptors that bind other **platelets to mediate aggregation** (for example, GP IIb/IIIa), and receptors for chemical signals such as **ADP, thrombin, and thromboxane.**

- The platelet surface membrane is essential for the coagulation cascade **because many clotting factors require this phospholipid surface to become functionally active.**

- **Platelets** contain distinct granules that are released upon activation.

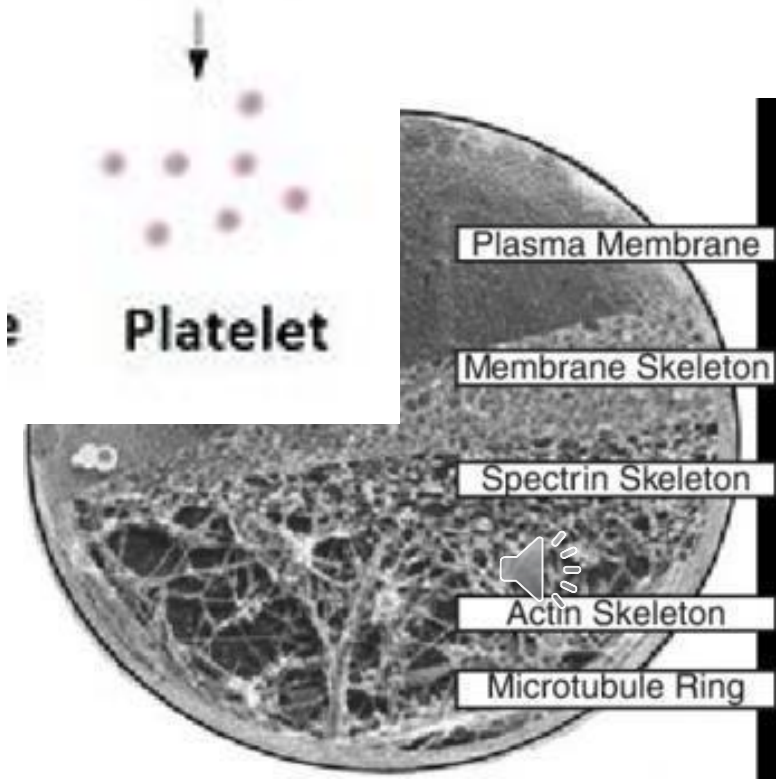


Platelets are a major player

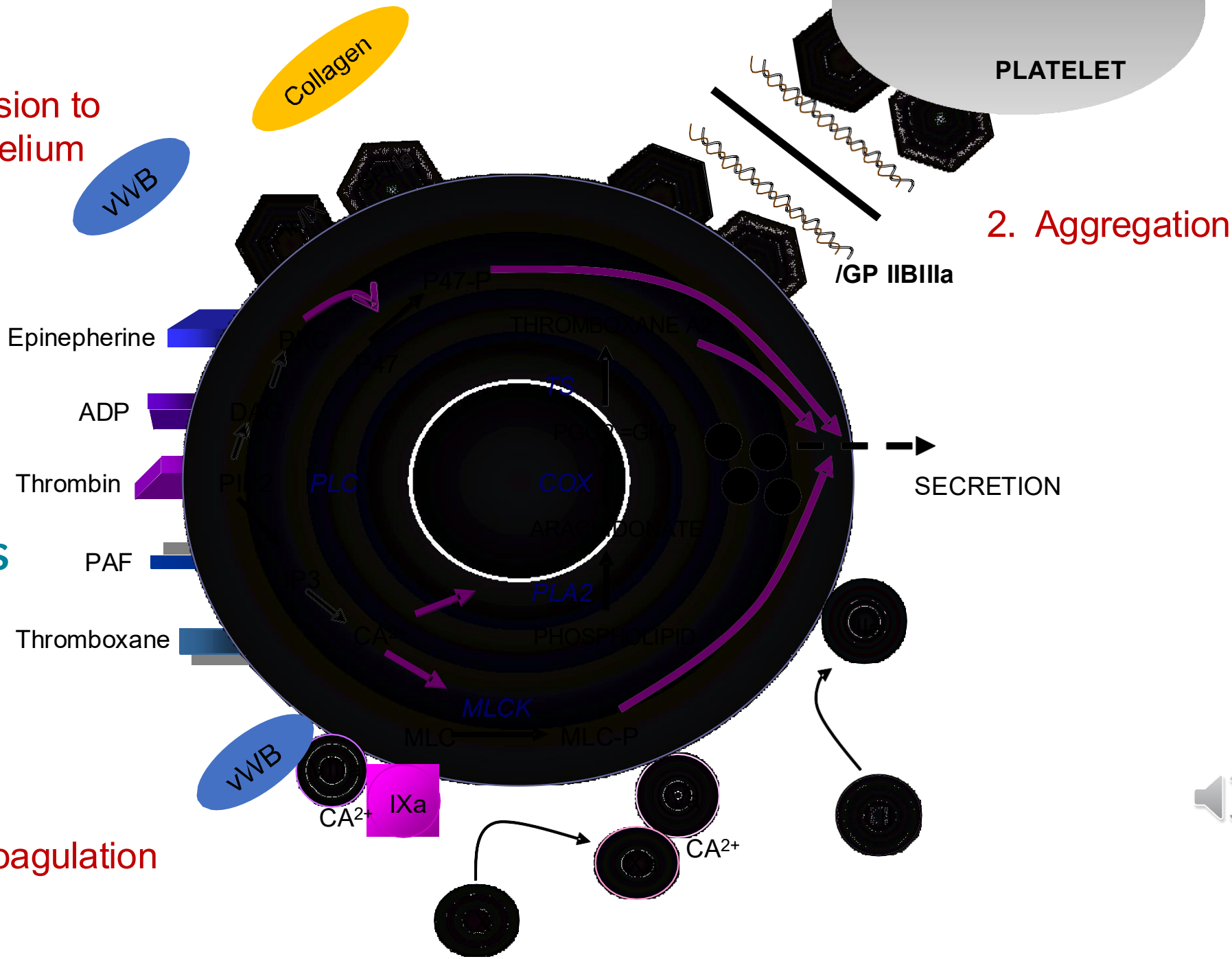
- Small anuclear cell fragments produced from the megakaryocytes.
- Platelets have numerous kinds of surface receptors. That affects or rather dictates its function. Different receptors correspond to different granules.
- Platelets also have actin filaments and myosin, which change the shape of the platelet upon activation.
- They also have three types of granules that store substances that are released upon platelet activation.



Megakaryocyte



1. Adhesion to endothelium



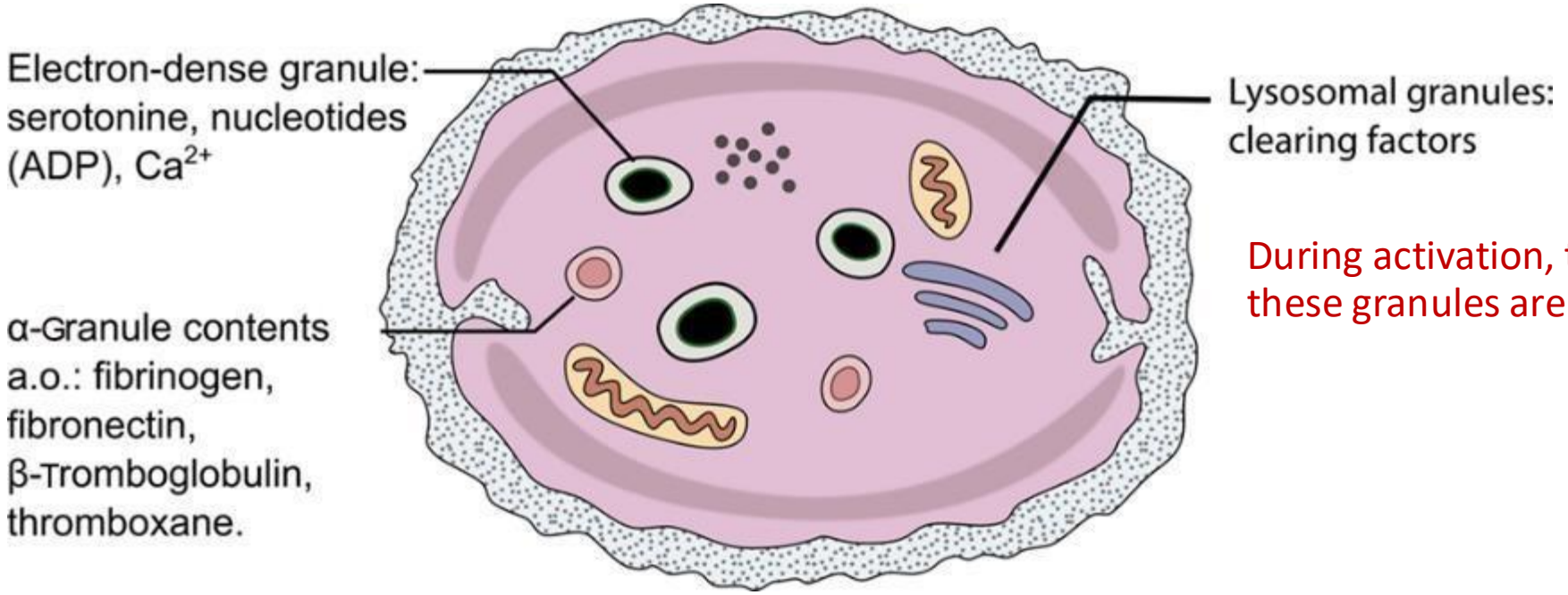
We'll discuss these receptors and signaling pathways later in detail.

3. Coagulation

The granules

You don't really have to memorize the different classes of granules as the doctor won't ask us to list them but the contents will be discussed(and reqiured) later.

- Electron-dense granules (calcium ions, ADP, ATP, serotonin)
- α-granule (a heparin antagonist, platelet-derived growth factor, fibrinogen, von Willebrand factor (vWF), clotting factors)
- Lysosomal granules (hydrolytic enzymes)



During activation, the contents of these granules are secreted.

- Important notes:

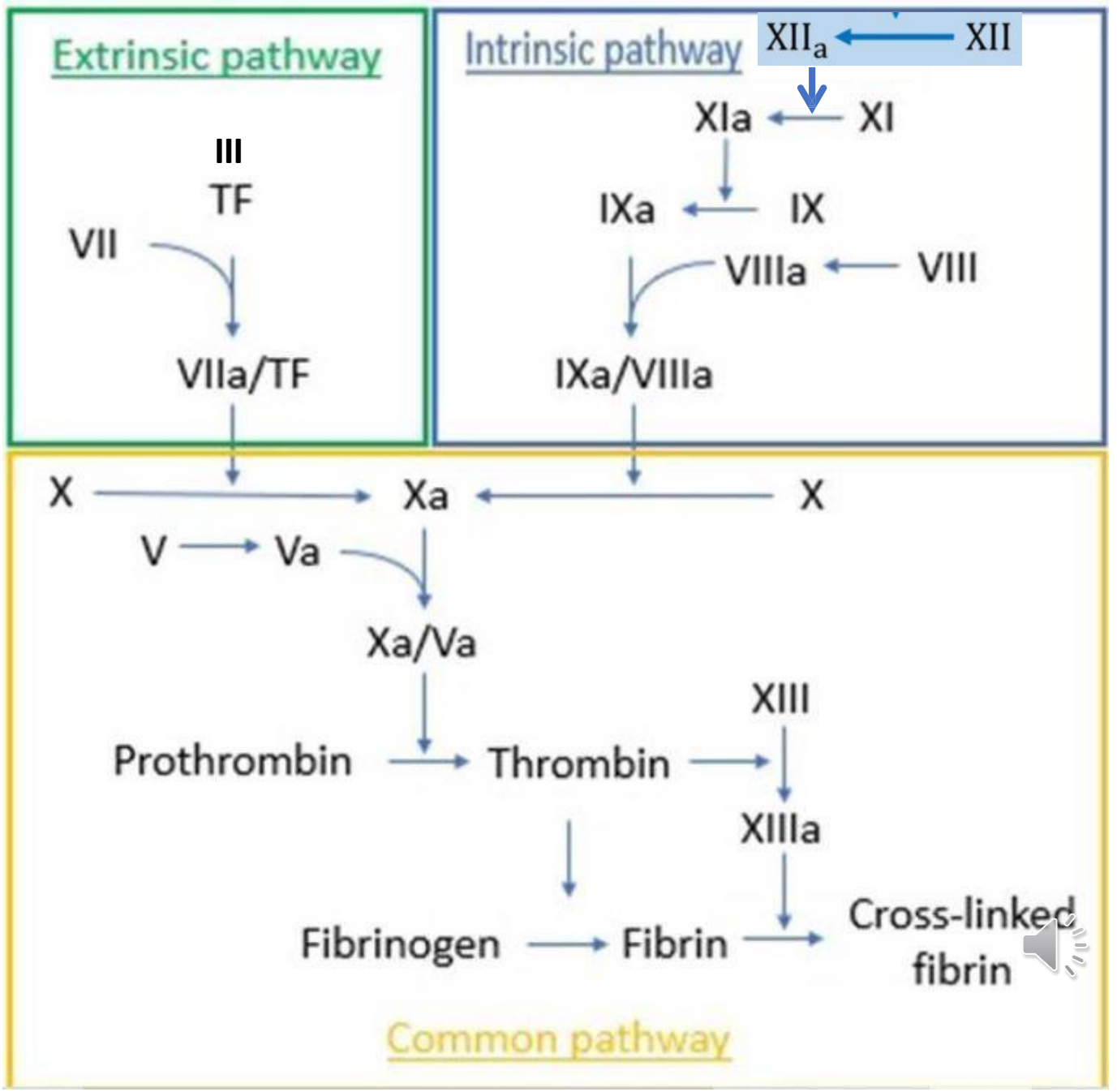
1 The activation of the intrinsic pathway requires binding to collagen or a negative surface such as some plastic tubes.

2 Since collagen is present in the endothelium, a tiny ***internal*** damage such as hypertension or diabetes can expose it, thus the name intrinsic pathway.

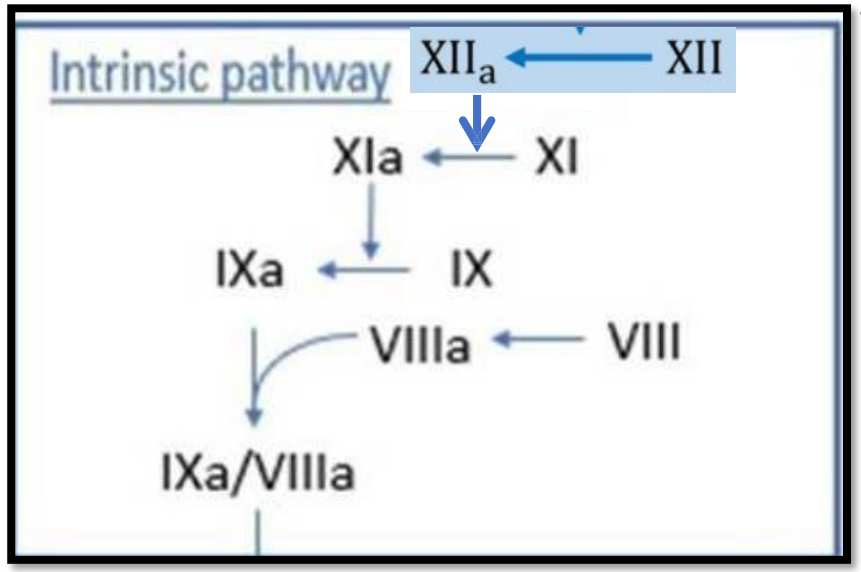
3 The activation of the extrinsic pathway requires a deeper injury exposing subendothelial tissue where the tissue factor is present. Usually such a deeper injury requires an external intervention such as knife cut.

4 Factor 3 is the tissue factor.

5 Factors 8 and 5 are co-factors for factors 9 and 10 respectively.



The purpose of the coagulation cascade is to strengthen the clot.



- As you know there are two different coagulation pathways:

1) The intrinsic pathway
2) The extrinsic pathway

The intrinsic pathway

it starts from within the blood vessel meaning the problem/trigger originates in the blood (hypertension, diabetes, oxidizing agents) not in the surrounding tissue, these intrinsic triggers damage the innermost layer of the blood vessels which is the endothelium exposing the underlying collagen fibers once exposed the intrinsic pathway gets activated.

1. the 12th coagulation factor is activated after binding to the exposed collagen or to a negatively charged blood tube.
2. The activated 12th factor is a serine protease, which is the case for many of these factors.
3. After it is activated it goes on to activate the 11th factor
4. which then proceed to activate the 9th factor via its proteolytic activity.

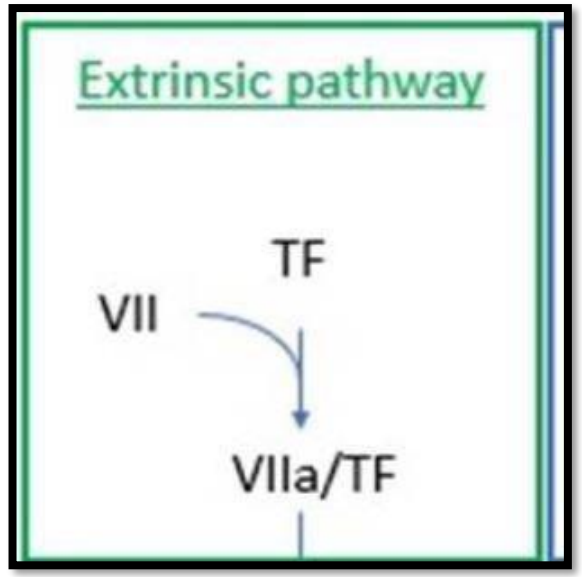
5. Now the activated 9th factor needs the 8th factor in order for it to be completely active so they interact with eachother forming an active complex.

The Extrinsic pathway:

1. Extrinsic Pathway – Step by Step :

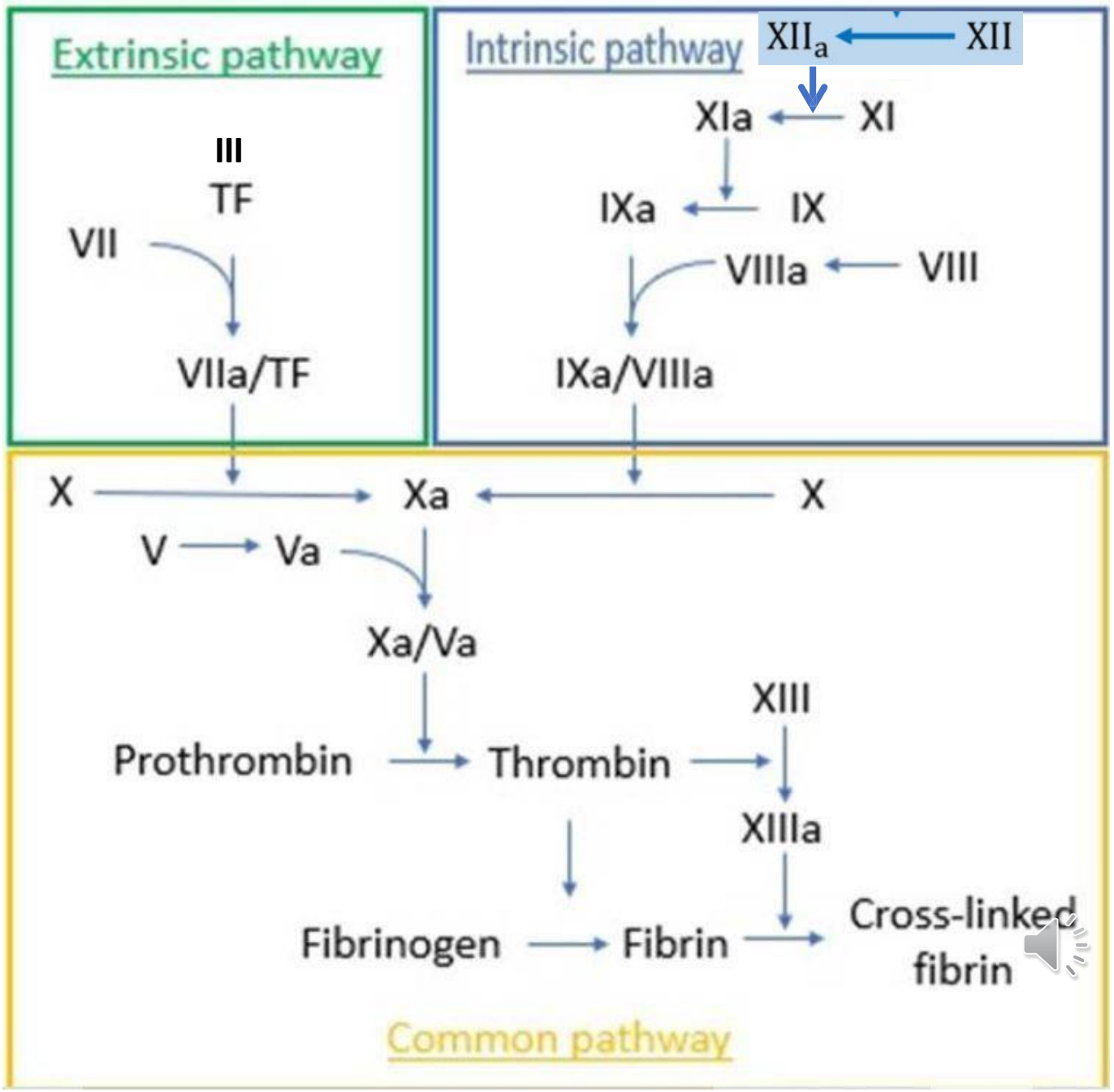
- **Deep external tissue injury** occurs, exposing **Tissue Factor (Factor III)**.
- **Factor VII binds to Tissue Factor (Factor III)**.
- Factor VII becomes activated: **Factor VII → Factor VIIa**.
- The **Tissue Factor-Factor VIIa complex (TF-VIIa)** forms.
- This complex activates **Factor X → Factor Xa**.
- The extrinsic pathway therefore leads to the activation of Factor X, initiating the common pathway.

III



❖ **Common Pathway ::**

1) **Factor X is activated to Factor Xa** by either the intrinsic or extrinsic pathway.
2) **Factor Xa requires activated Factor V (Factor Va) as a cofactor.**
3) Factor Xa combines with Factor Va to form the **Xa-Va complex**, called the *prothrombinase complex*.
4) This complex converts **Prothrombin (Factor II) → Thrombin (Factor IIa).**
5) Thrombin converts **Fibrinogen into Fibrin**, which contributes to blood clot formation.



- **The Major Players and Requirements:** The core active factors (Factors II, VII, IX, and X) strictly require three elements to function:

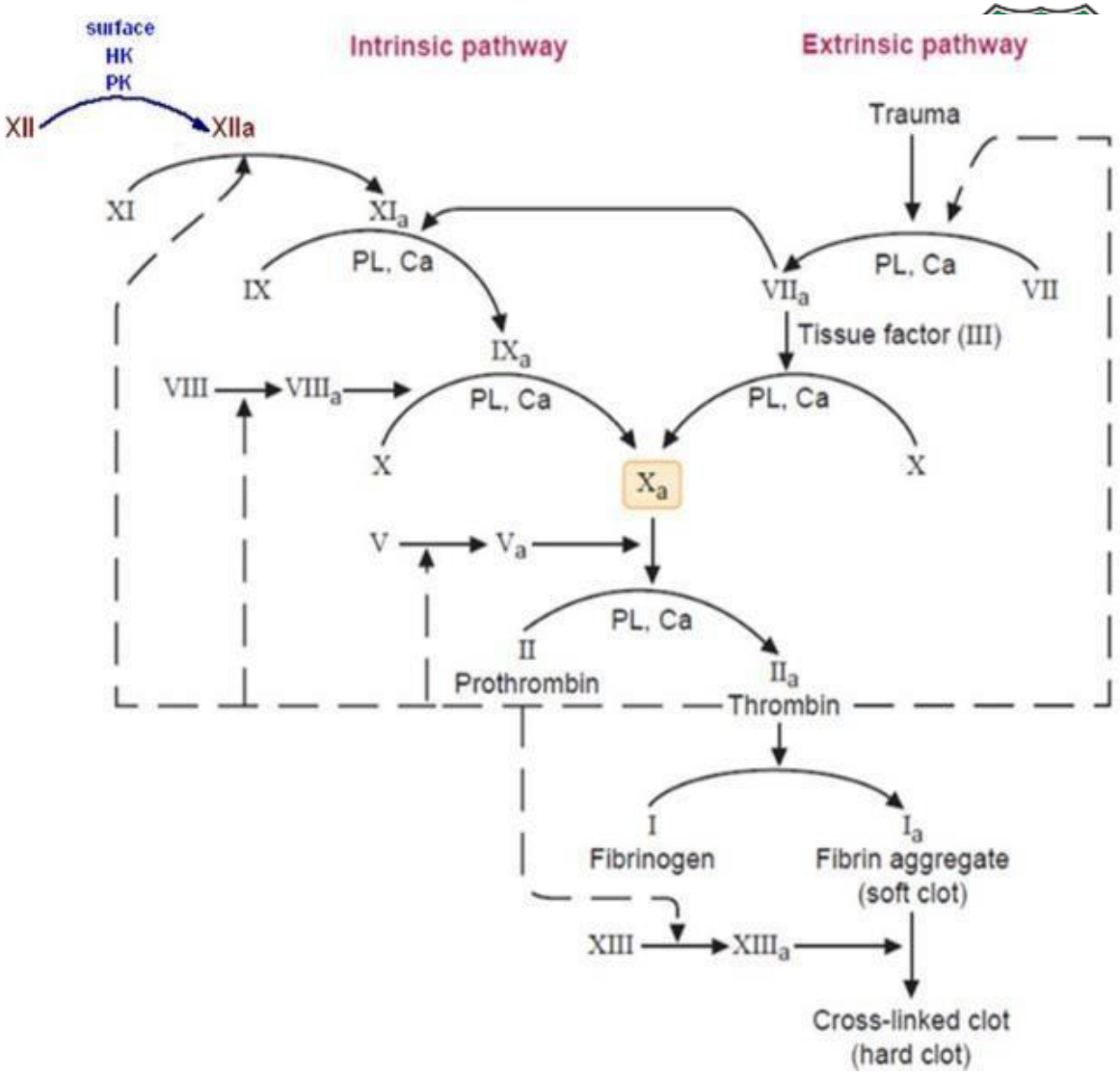
- **Calcium ions**, **Vitamin K**, and the **phospholipid surface of the platelets.**

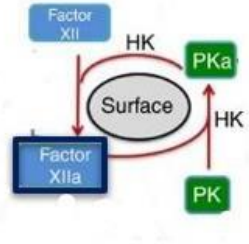
- **Pathway Crosstalk:** The extrinsic and intrinsic pathways are heavily intertwined. Notably, Factor VIIa from the extrinsic pathway **jumps over** and activates **Factor IX** in the intrinsic pathway, demonstrating significant communication between the cascades.

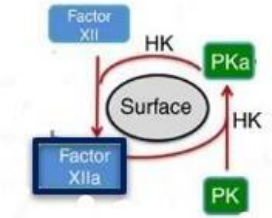
- **Factor XII Positive Feedback:** When Factor XII binds to collagen and becomes activated, it activates a molecule called **Prekallikrein** into **Kallikrein**. Kallikrein then loops back and further activates more Factor XII, creating a continuous **positive feedback** loop.

- **Thrombin Positive Feedback:** To ensure rapid thrombus formation, Thrombin (Factor IIa) exerts a massive positive feedback loop on multiple upstream factors.

·Thrombin activates Factors VII and XI.
·Thrombin also activates the critical cofactors, Factors V and VIII







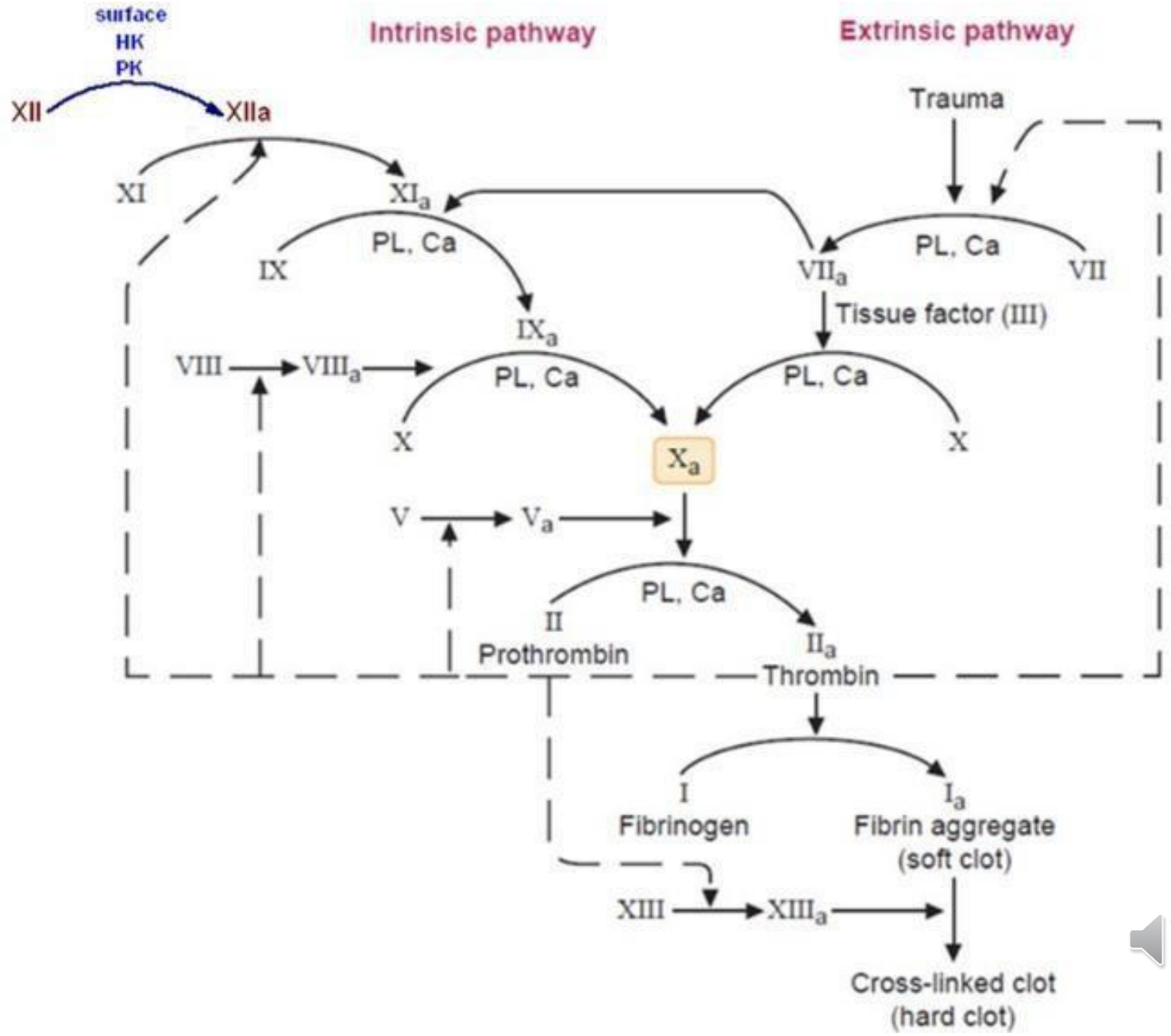
- Important notes:

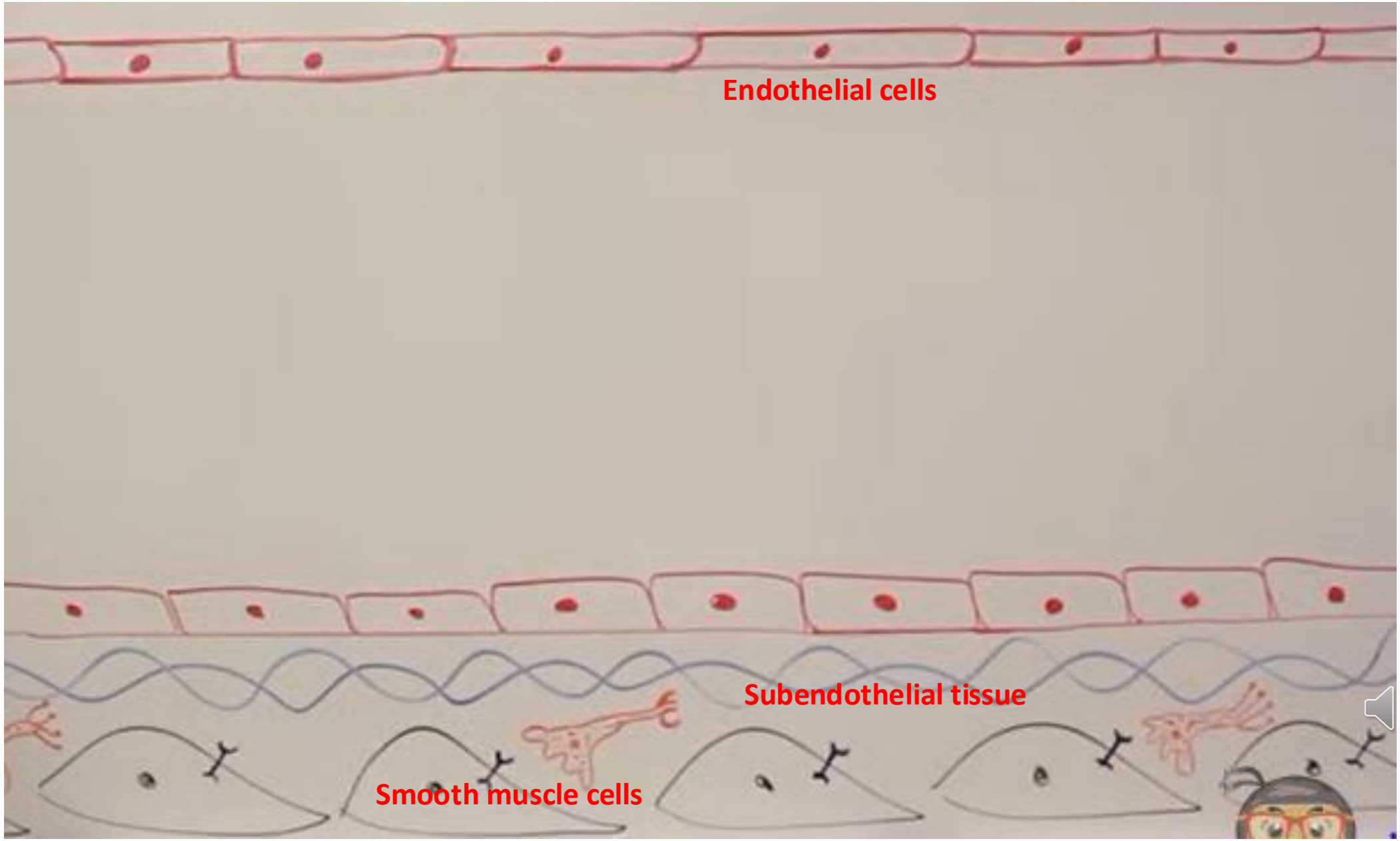
1 Factors 2,7,9 and 10 (the major players) require Ca+2, vitamin K and platelets surface.

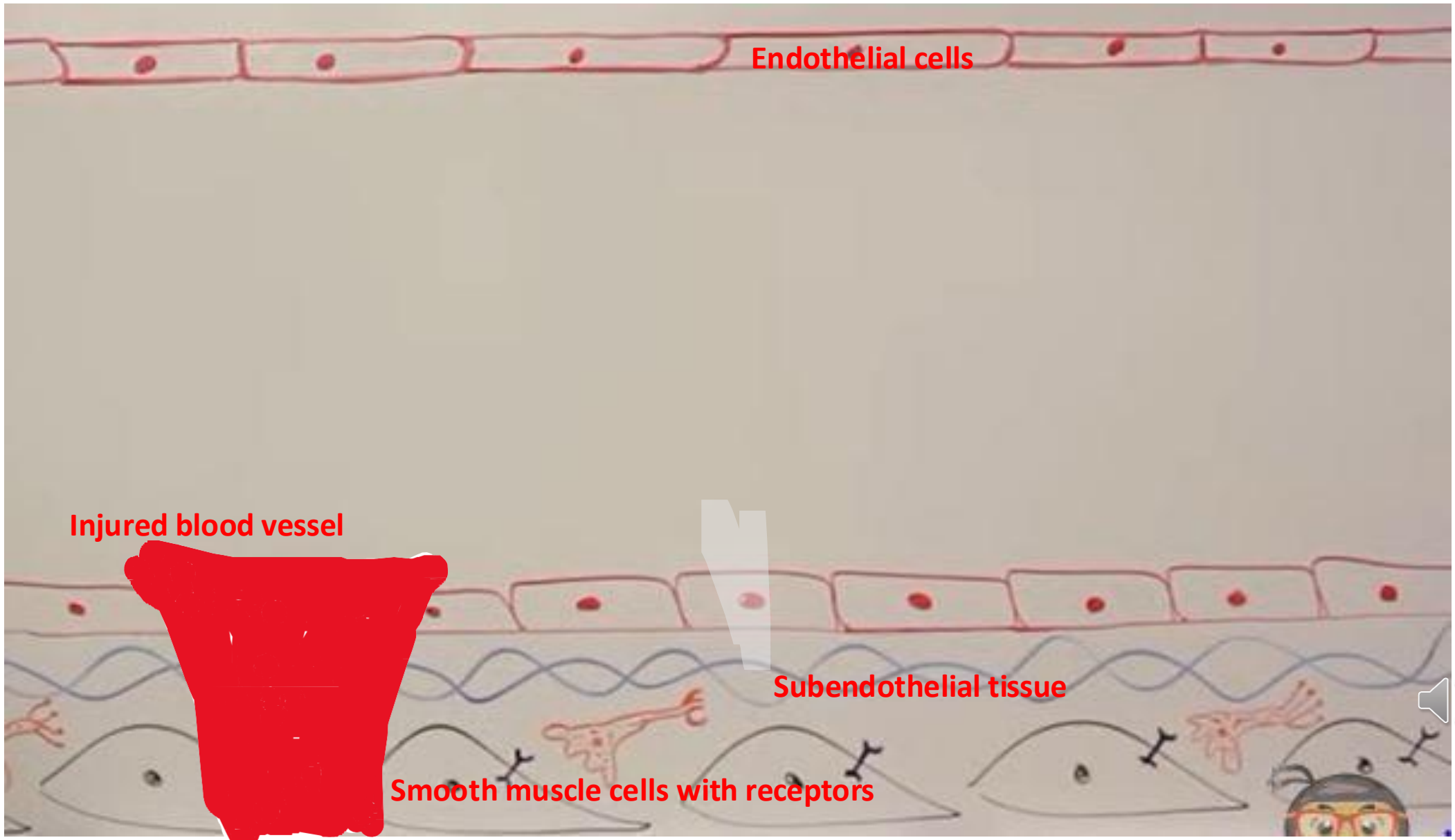
2 Notice the connection between factor 7 and 9, connecting the extrinsic pathway to the intrinsic pathway.

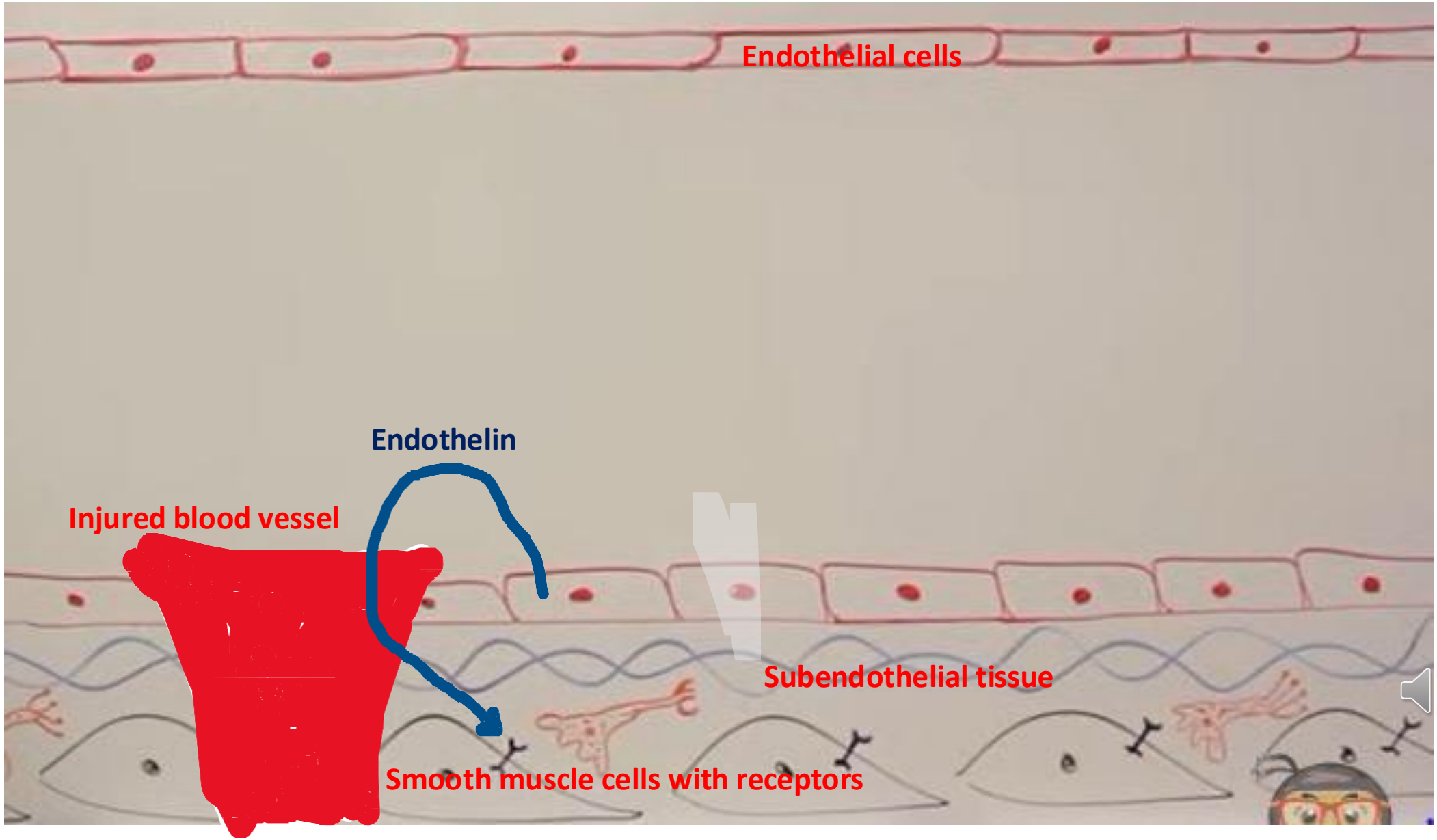
3 Thrombin (factor 2): Does a positive feedback loop to factors 7,11, 5 and 8.

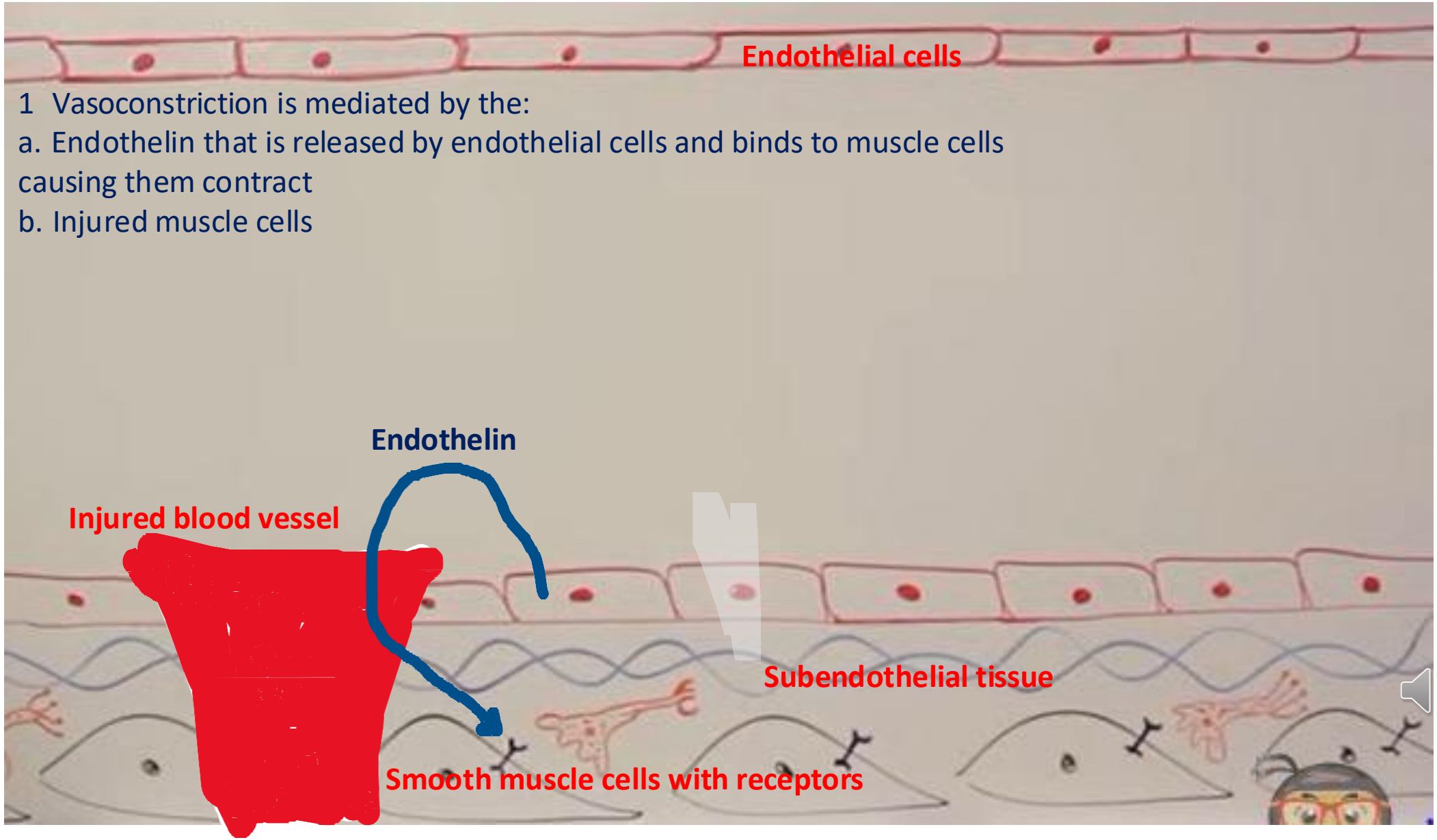
4 Factor 12 performs a positive feedback loop with Prekallekrein and activates it to Kallekrein, which itself reactivates factor 12.

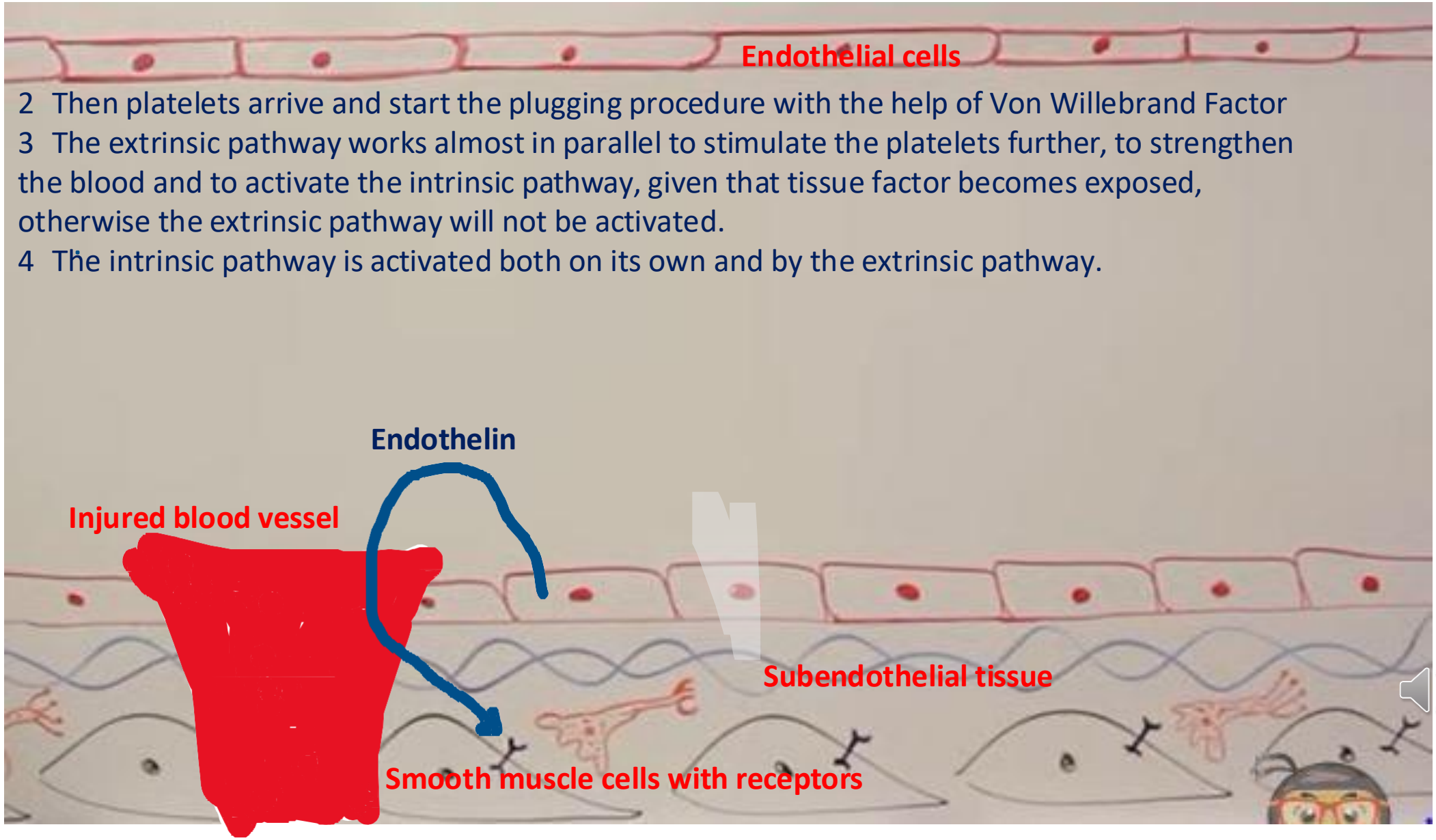



Endothelial cells
Subendothelial tissue
Smooth muscle cells


Endothelial cells
Injured blood vessel
Subendothelial tissue
Smooth muscle cells with receptors


Endothelial cells
Endothelin
Injured blood vessel
Subendothelial tissue
Smooth muscle cells with receptors


Endothelial cells
1 Vasoconstriction is mediated by the:
a. Endothelin that is released by endothelial cells and binds to muscle cells causing them contract
b. Injured muscle cells
Endothelin
Injured blood vessel
Subendothelial tissue
Smooth muscle cells with receptors


Endothelial cells
2 Then platelets arrive and start the plugging procedure with the help of Von Willebrand Factor
3 The extrinsic pathway works almost in parallel to stimulate the platelets further, to strengthen the blood and to activate the intrinsic pathway, given that tissue factor becomes exposed, otherwise the extrinsic pathway will not be activated.
4 The intrinsic pathway is activated both on its own and by the extrinsic pathway.
Endothelin
Injured blood vessel
Subendothelial tissue
Smooth muscle cells with receptors

Hemostasis: What Happens After Vessel Injury?

1. **These processes happen almost simultaneously**

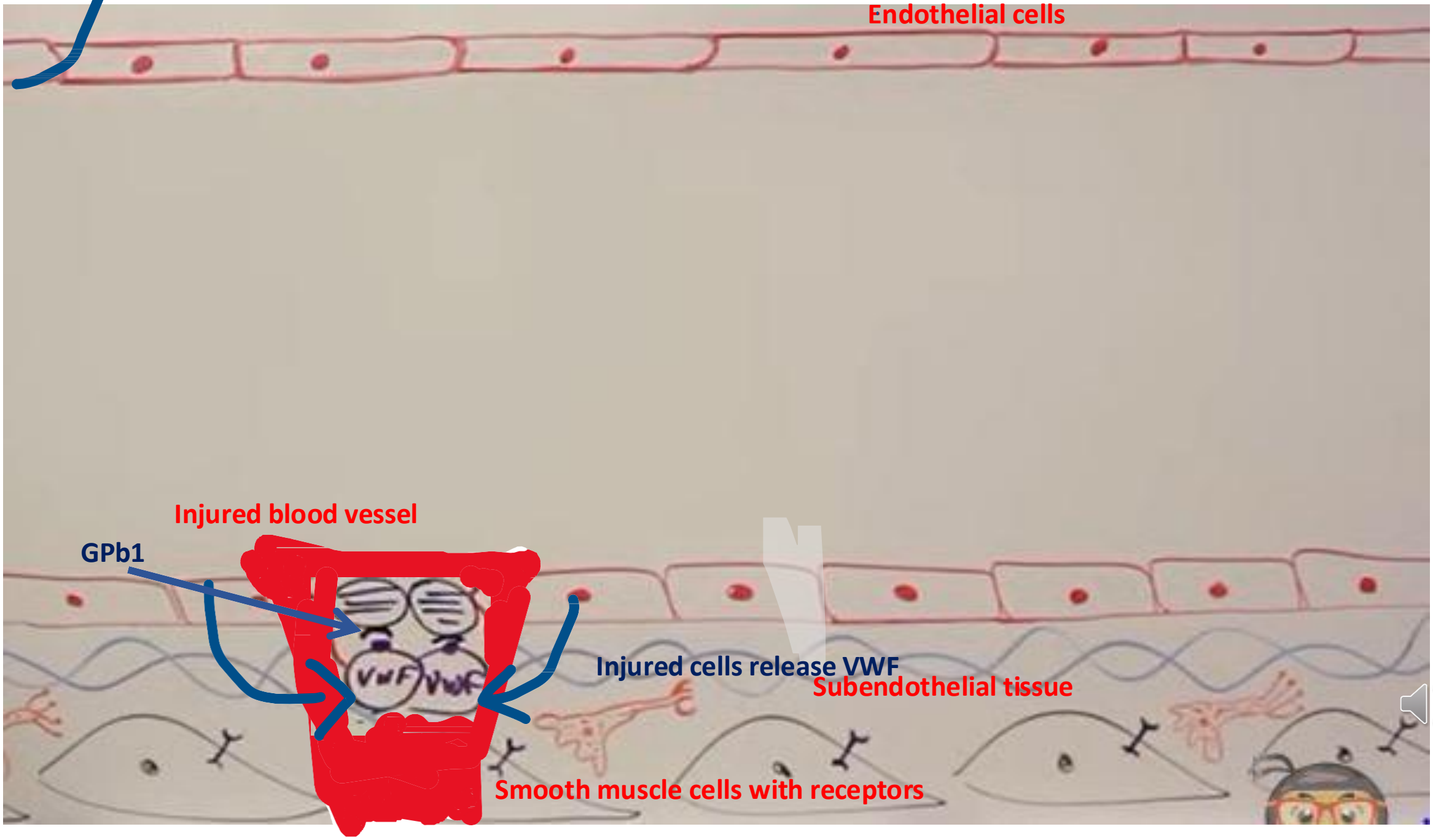
- When a blood vessel is injured, three processes begin at nearly the same time:
- **Vasoconstriction:** The blood vessel narrows.
- **Platelet recruitment:** Platelets gather at the injured site.
- **Coagulation cascade activation:** Clotting factors become activated to help form a clot.

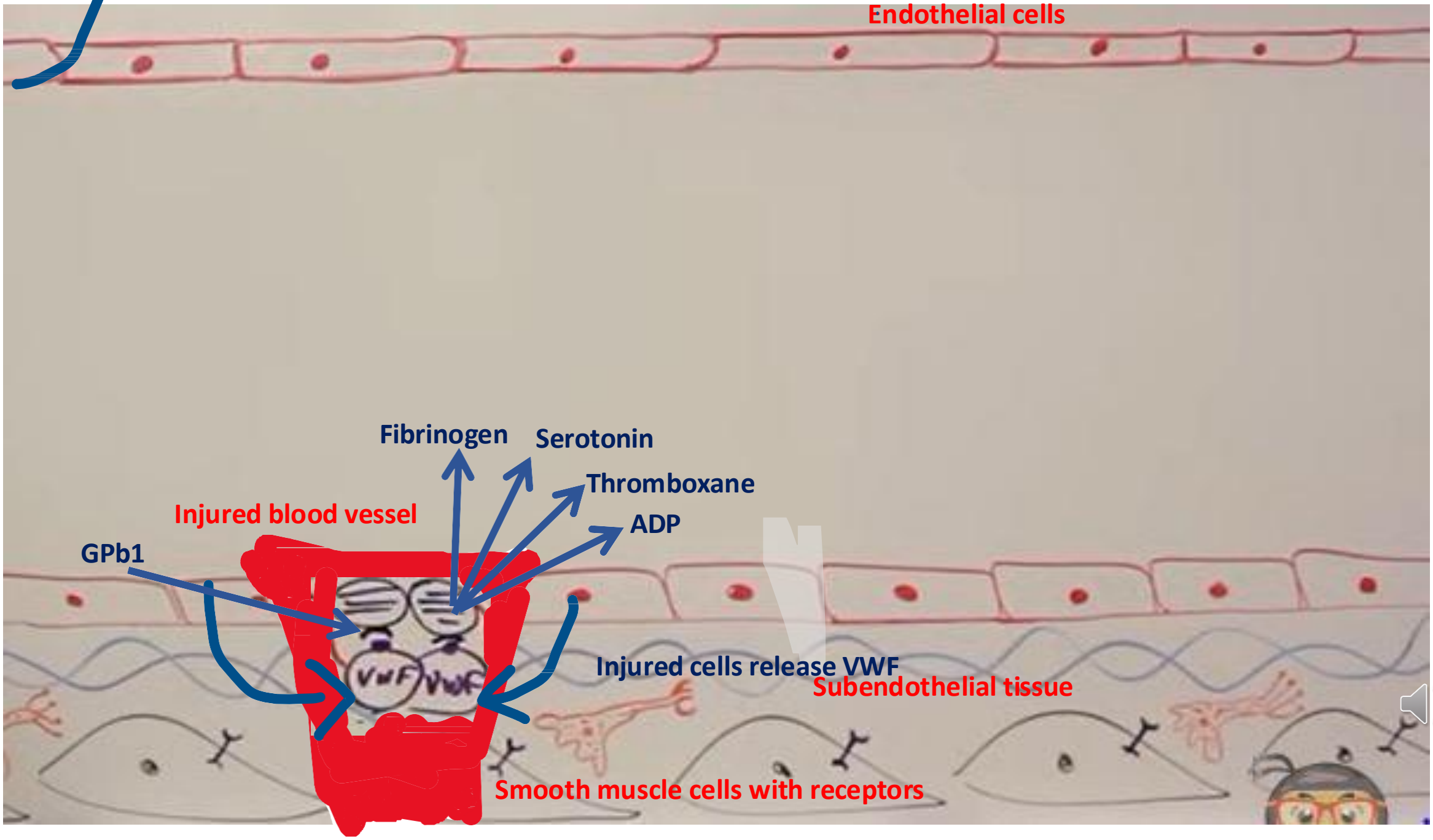
2. **How does vasoconstriction happen?**

- Damaged endothelial cells release **endothelin**.
- Endothelin binds to receptors on the smooth muscle cells beneath the endothelium, causing them to contract.
- Smooth muscle cells directly damaged by the injury also contract.
- Together, these contractions narrow the blood vessel and reduce blood loss.

3. **How are platelets and coagulation activated?**

- Platelets immediately begin gathering at the site of injury.
- If the injury exposes **subendothelial collagen**, it triggers the **intrinsic pathway**.
- If the injury is deep enough to expose **tissue factor (Factor III)** in the underlying tissue, it triggers the **extrinsic pathway**.


Endothelial cells
Injured blood vessel
GPb1
VWF
VWF
Injured cells release VWF
Subendothelial tissue
Smooth muscle cells with receptors


Endothelial cells
Fibrinogen
Serotonin
Thromboxane
ADP
Injured blood vessel
GPb1
VWF
VWF
Injured cells release VWF
Subendothelial tissue
Smooth muscle cells with receptors

Endothelial cells

The signals lead to platelet aggregation and change in their shape

Fibrinogen

Serotonin

Thromboxane

ADP

Injured blood vessel

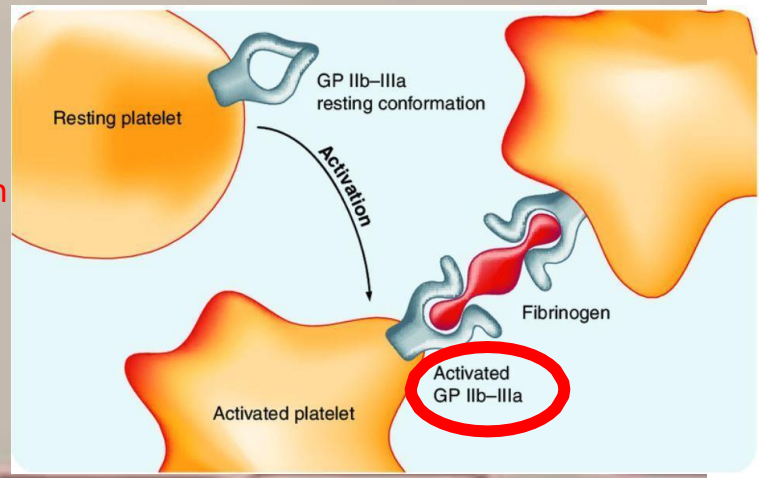
GPb1

VWF VWF

Injured cells release VWF

Subendothelial tissue

Smooth muscle cells with receptors



Endothelial cells

-Platelets recruitment: Injured tissues release the **Van Wilderband Factor (VWF)** which is recognized by the platelets. The platelets then adhere to it using Glycoprotein 1b (GP1b).

-The adhered platelets then release ADP, thromboxane, serotonin and fibrenogen.

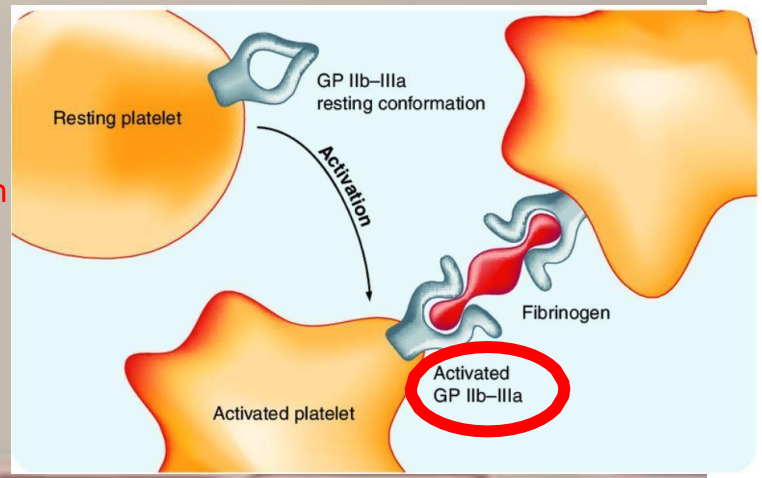
1 ADP is the ***key signal in aggregating the platelets*** and is recognized by G proteins P2Y1 and P2Y12 receptors on the other platelets. This signal upregulates proteins GP2b and GP3a on the surface of the platelets allowing them to bind to each others (aggregate). The signal also induces the platelets to change their shape (somewhat contract) which increases their potential to release more granules of ADP, Ca+2, serotonin and thromboxane. Clopidogrel (Plavix) inhibits the P2Y receptors, thus it is an anticoagulant.

2 Thromboxane also activates other platelets to aggregate, and it works as vasoconstrictor.

3 Serotonin works as vasoconstrictor mainly.

4 Fibrinogen connects the platelets together.

The signals lead to platelet aggregation and change in their shape



Fibrinogen

Serotonin

Thromboxane

ADP

Injured blood vessel

GPb1

VWF VWF

Injured cells release VWF

Subendothelial tissue

Smooth muscle cells with receptors

❑ Primary Platelet Adhesion

1. Injured endothelial cells release **von Willebrand factor (vWF)**.
2. Circulating platelets recognize vWF and bind to it through their **glycoprotein Ib (GP1b) receptors**.
3. This interaction anchors platelets to the site of injury.

❑ Platelet Activation and Secretion

1. Binding to vWF activates the platelets.
2. Activated platelets **change shape** and release their granular contents, including:

I. Fibrinogen
II. Serotonin
III. Thromboxane
IV. ADP

❑ The Crucial Role of ADP

- **ADP is the most important signal for recruiting additional platelets** to the injury site.

1. ADP binds to **P2Y receptors** on nearby resting platelets.
2. This activates intracellular signaling, increasing the expression of **glycoprotein IIb/IIIa (GP2b/3a) receptors** on the platelet surface.
3. These receptors provide binding sites for fibrinogen.
4. **Fibrinogen acts as a bridge** between GP2b/3a receptors on adjacent platelets, allowing them to stick together and form a platelet aggregate.

❑ Pharmacological Application: Clopidogrel (Plavix)

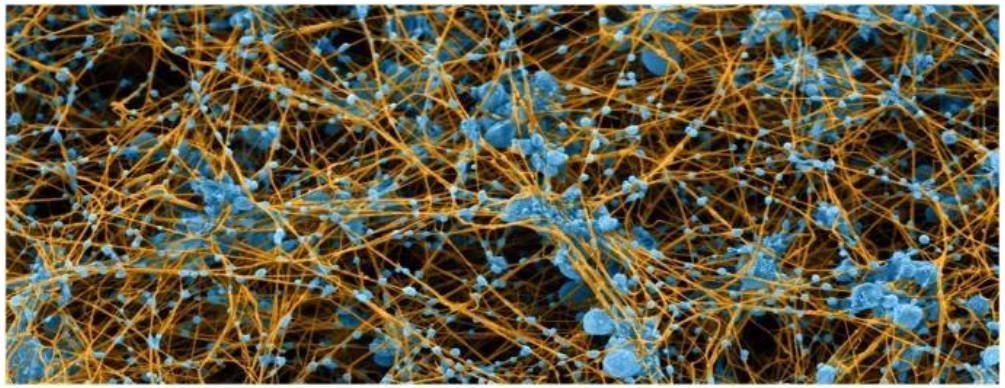
- **Clopidogrel (Plavix)** inhibits the platelet ADP receptor (P2Y), preventing ADP-mediated platelet activation and aggregation.

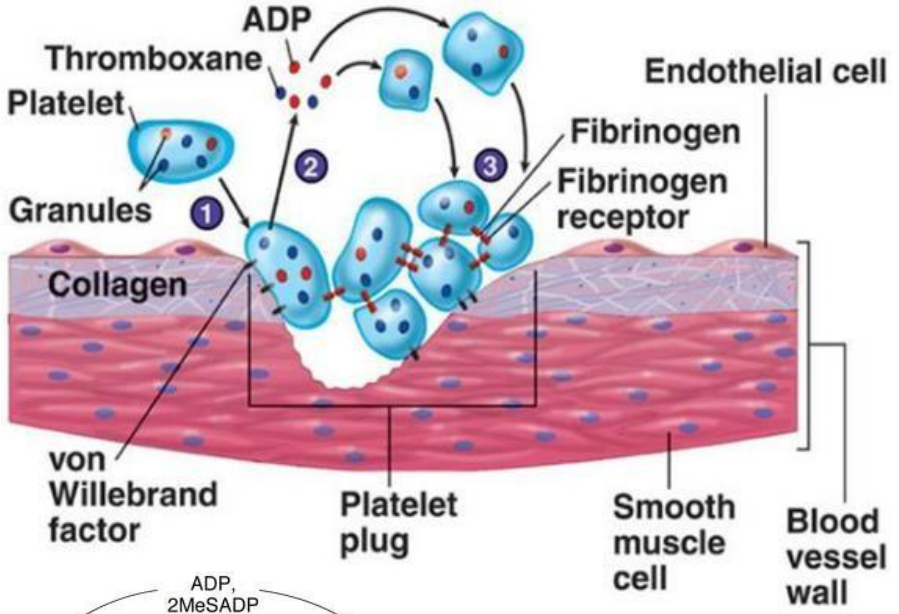
❑ Functions of Other Secretions

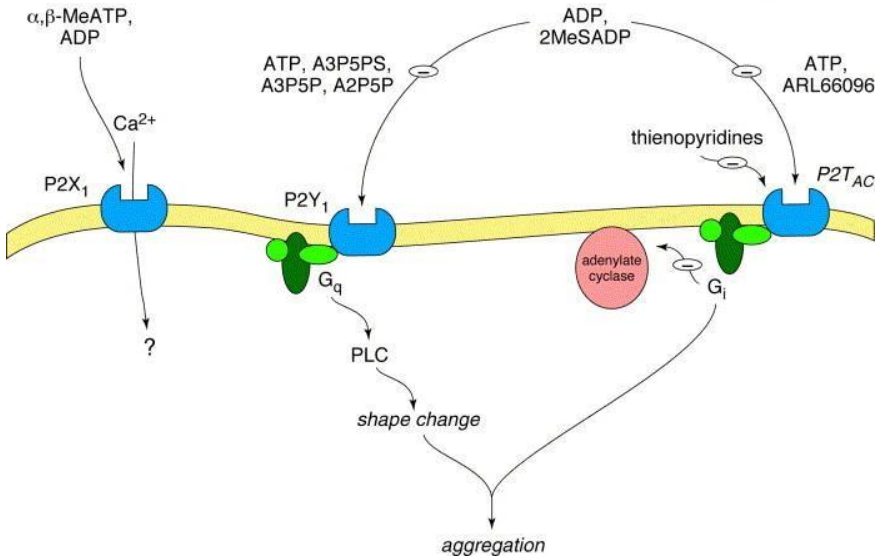
- **Thromboxane:** Promotes platelet aggregation, although less powerfully than ADP, and causes vasoconstriction.
- **Serotonin:** Primarily promotes further vasoconstriction.
- **Fibrinogen:** Acts as a physical bridge connecting adjacent platelets during aggregation.

ADP drives the formation of platelet plug

- ADP is a platelet activator that binds to its receptor and modifies the platelet membrane allowing fibrinogen to adhere to platelet surface glycoproteins resulting in fibrinogen-induced platelet aggregation, called platelet plug.







Granules released by the platelets

- Some platelets release substances from the granules:
  - ADP
  - Serotonin
  - Factor V
  - ATP
  - Calcium
  - Fibrinogen
  - vWF
  - Thrombin
  - Thromoxane

The ones in red are the ones that are produced in high amounts by the platelets.

1. Morphological Changes

- ADP and vWF activate platelets.
- The platelets lose their normal, smooth shape.
- They develop **pseudopodia** – tentacle-like projections that help them interact with other platelets and the injured surface.

2. Important Granule Contents

- Activated platelets release several substances, including **ATP, calcium, and Factor V**.
- However, the main high-yield molecules involved in immediate platelet plug formation are:

I. **ADP:** Recruits and activates additional platelets.
II. **Serotonin:** Promotes vasoconstriction.
III. **Fibrinogen:** Bridges adjacent platelets, promoting aggregation.
IV. **Thromboxane:** Promotes platelet aggregation and vasoconstriction.

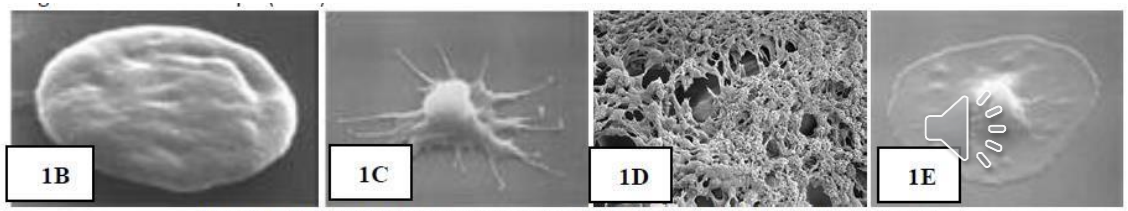
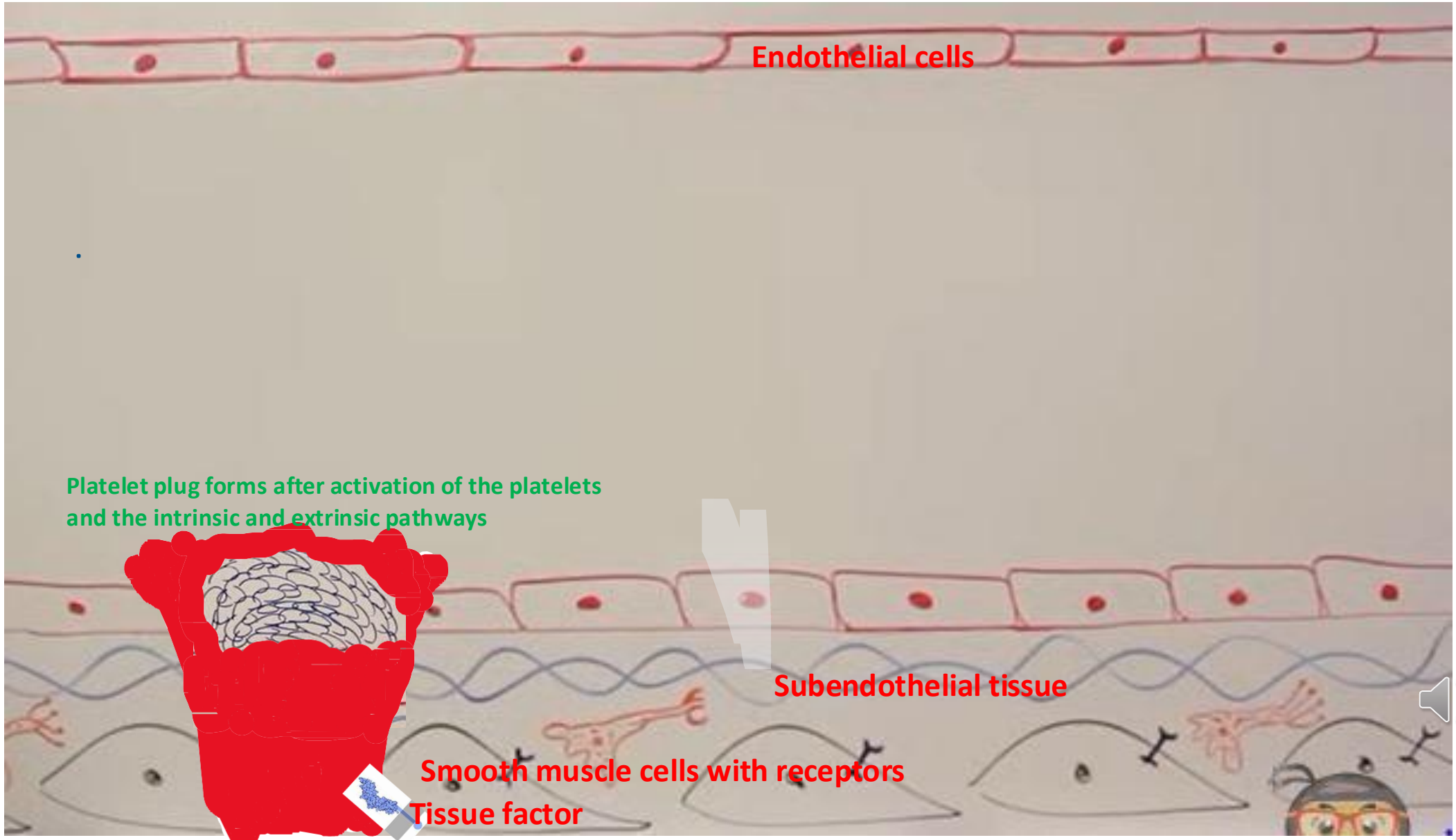


Figure 1 [(**B**) Platelet in resting mode (**C**) Activated platelets change into a pseudopodia shape (**D**) Aggregated platelets (**E**) Platelet spreading]

- Platelets also change their shape allowing for more platelet-platelet interaction and aggregation.


Endothelial cells
Platelet plug forms after activation of the platelets
and the intrinsic and extrinsic pathways
Subendothelial tissue
Smooth muscle cells with receptors
Tissue factor

Endothelial cells

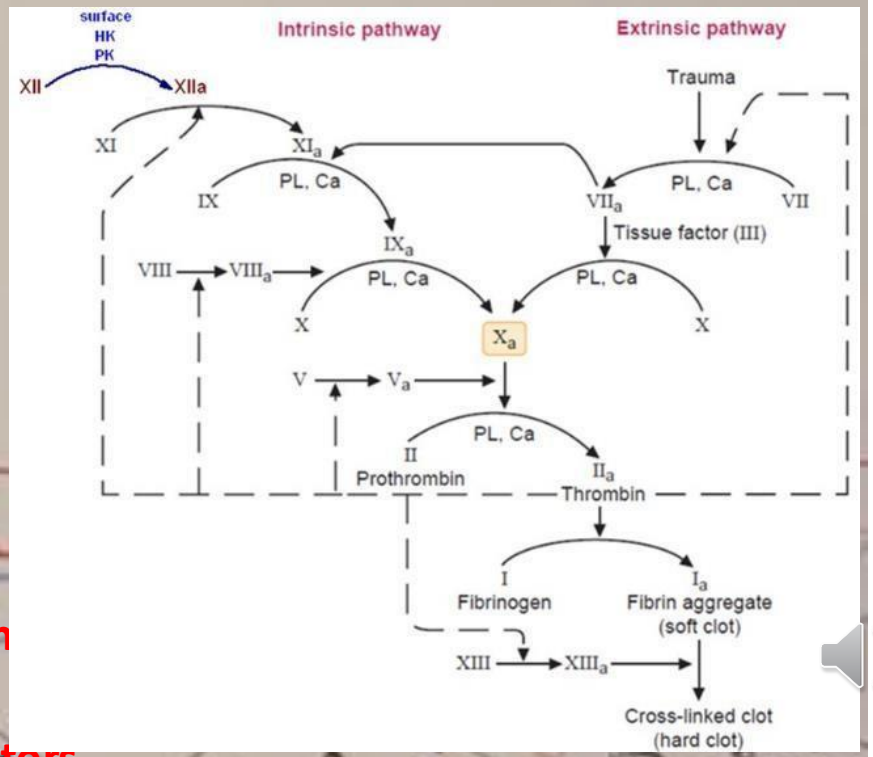
Platelet plug forms after activation of the platelets and the intrinsic and extrinsic pathways



Suben

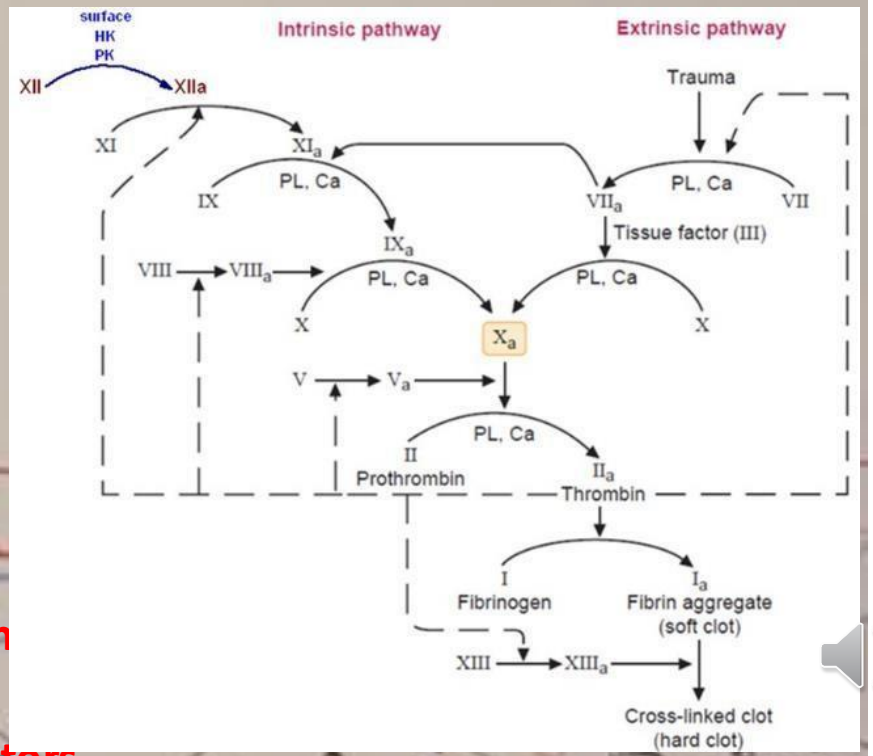
Smooth muscle cells with receptors

Tissue factor



Endothelial cells

- In parallel to platelet activation, the extrinsic and the intrinsic pathways are also activated. The extrinsic pathway is activated only if the tissue factor is exposed. The extrinsic pathway is activated in approximately 10 seconds. The intrinsic pathway is activated in 4-6 minutes.
- If the extrinsic pathway is activated then factor VII activates factor 9 from the intrinsic pathway. This along with factor 10 are the connection between both pathways.
- The activation of both pathways causes the activation of thrombin which further activates the platelets to aggregate and induces them to release their granules.
- Thrombin itself further does positive feedback on factors 7,11, 5 and 8, so this amplifies the process very fast.



Platelet plug forms after activation of the platelets and the intrinsic and extrinsic pathways



Suben

Smooth muscle cells with receptors

Tissue factor

1. Timeline of Cascade Activation

- **Extrinsic pathway:** The rapid-response pathway. It can activate in approximately **10 seconds** when a deep wound exposes tissue factor.
- **Intrinsic pathway:** A slower pathway, taking approximately **4–6 minutes** to fully activate on its own.

2. Two Sources of Thrombin

Platelets: Activated platelets provide a phospholipid surface that supports thrombin generation; they may also contribute to thrombin generation through platelet-associated mechanisms.

Coagulation cascade: The intrinsic and extrinsic pathways converge on the common pathway, generating thrombin (Factor IIa), which converts fibrinogen into fibrin to form a stable blood clot.

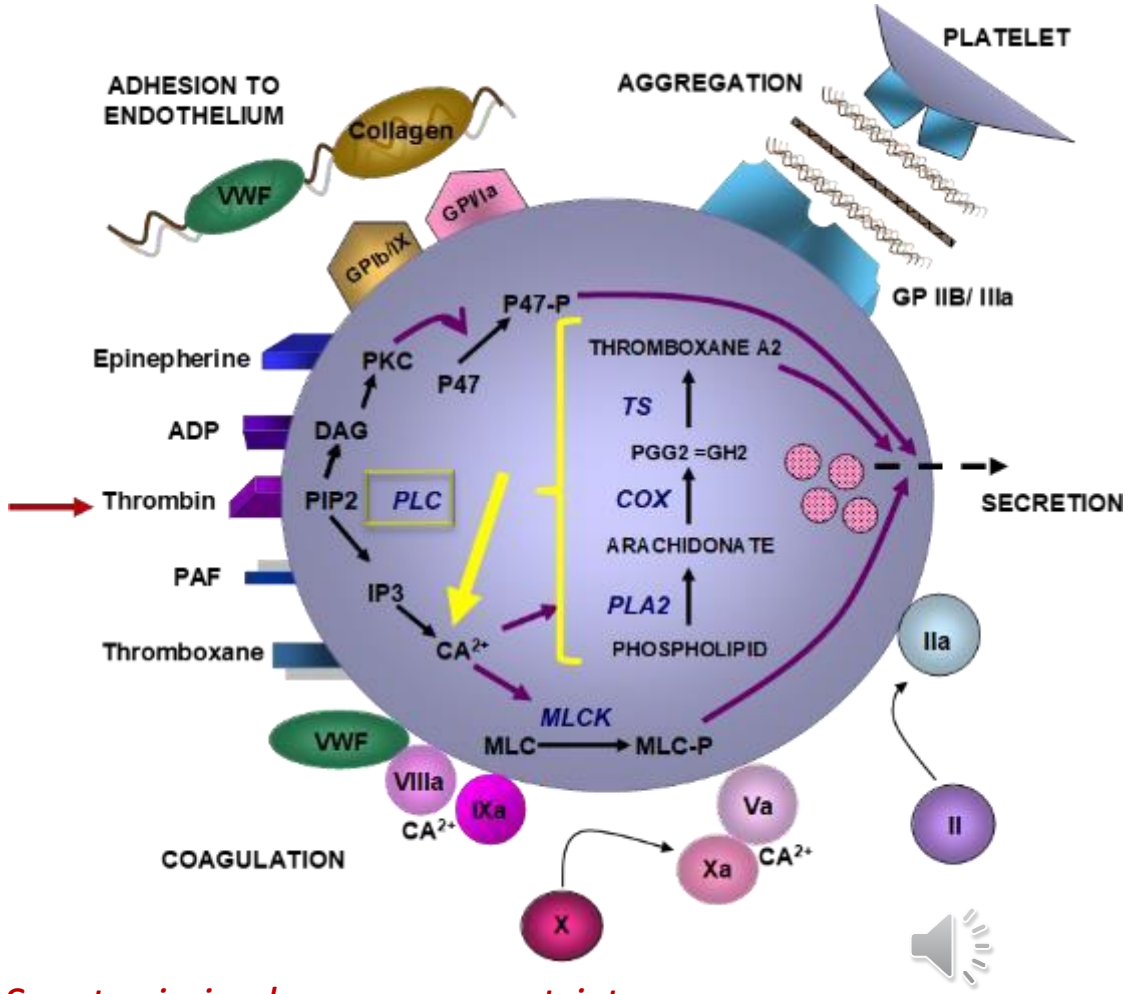
3. Thrombin's Role in Recruiting Platelets

- **ADP** recruits and activates the initial platelets at the injury site.
- The coagulation cascade produces a large burst of **thrombin**.
- Thrombin acts as a powerful additional signal, activating and recruiting many more platelets.
- This amplifies clot formation, increasing the clot's size and strengthening its integrity.

- **We will dive a little deeper into the role of thrombin in signaling to platelets. We will also discuss the coagulation cascade in more details.**

Thrombin signaling

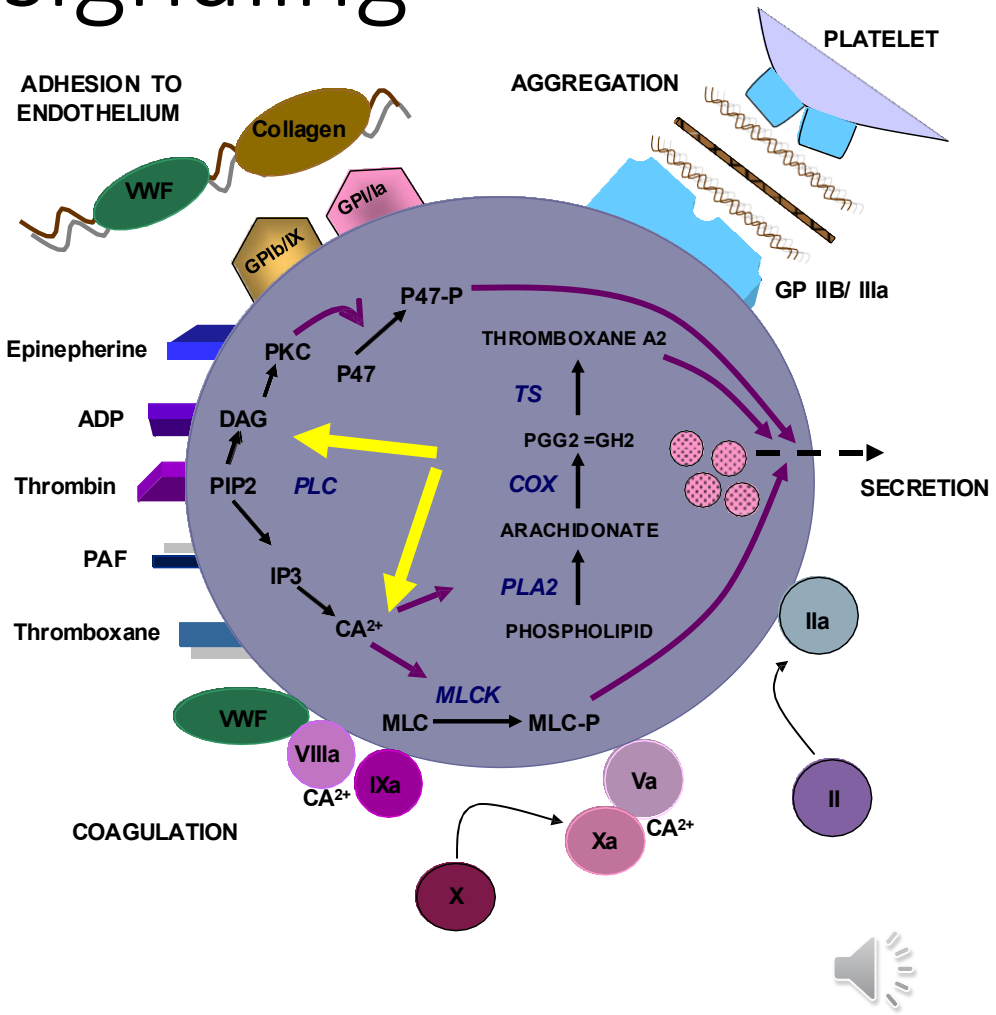
- ADP is a signal released by platelets to activate an aggregate other platelets. However, Thrombin is part of the coagulation cascade.
- The major function of thrombin is to activate fibrinogen, factor 13 among other functions which involve signaling to platelets.
- Thrombin receptor activates a G protein that activates phospholipase C-β (PLC-β).
- PLC-β hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG).
- IP3 induces the release of intracellular Ca^{2+} stores, and DAG activates protein kinase C (PKC).
- Ca^{2+} triggers the release of arachidonate from membrane phospholipids by phospholipase A2.
- Arachidonate is converted by cyclooxygenase to prostaglandins, which are then converted by thromboxane synthetase to thromboxane A2.
    - Thromboxane is as vasoconstrictor and a further inducer of PLC-β activity (and platelet aggregation).
    - It acts in autocrine and paracrine manners.



- *Serotonin is also a vasoconstrictor.*
- *PDGF stimulates proliferation of endothelial cells to reduce blood flow.*

More release of Thrombin signaling

- Ca^{2+} activates myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin allowing it to interact with actin and resulting in altered platelet morphology, induced motility, and release of granules.
- DAG activates PKC, which phosphorylates and activates specific platelet proteins that induce the release of platelet granule contents including ADP.



Thrombin Signaling in Platelets

1. Activation of Phospholipase C (PLC)

- Thrombin binds to its receptor on the platelet.
- This activates a **G protein**, which activates **Phospholipase C (PLC)**.

2. Formation of IP_3 and DAG.

3. PLC cleaves membrane phospholipids, producing two second messengers:

I. **IP_3 (Inositol Triphosphate)**
II. **DAG (Diacylglycerol)**

3. Calcium Release and Platelet Contraction

1) IP_3 travels to intracellular calcium stores and triggers the release of **Ca^{2+} into the cytoplasm**.
2) Ca^{2+} activates **Myosin Light-Chain Kinase (MLCK)**.
3) MLCK increases platelet contraction and motility, helping release platelet vesicles.

4. Protein Kinase C Activation

1) At the same time, DAG activates **Protein Kinase C (PKC)**.
2) PKC also promotes the release of internal platelet vesicles.

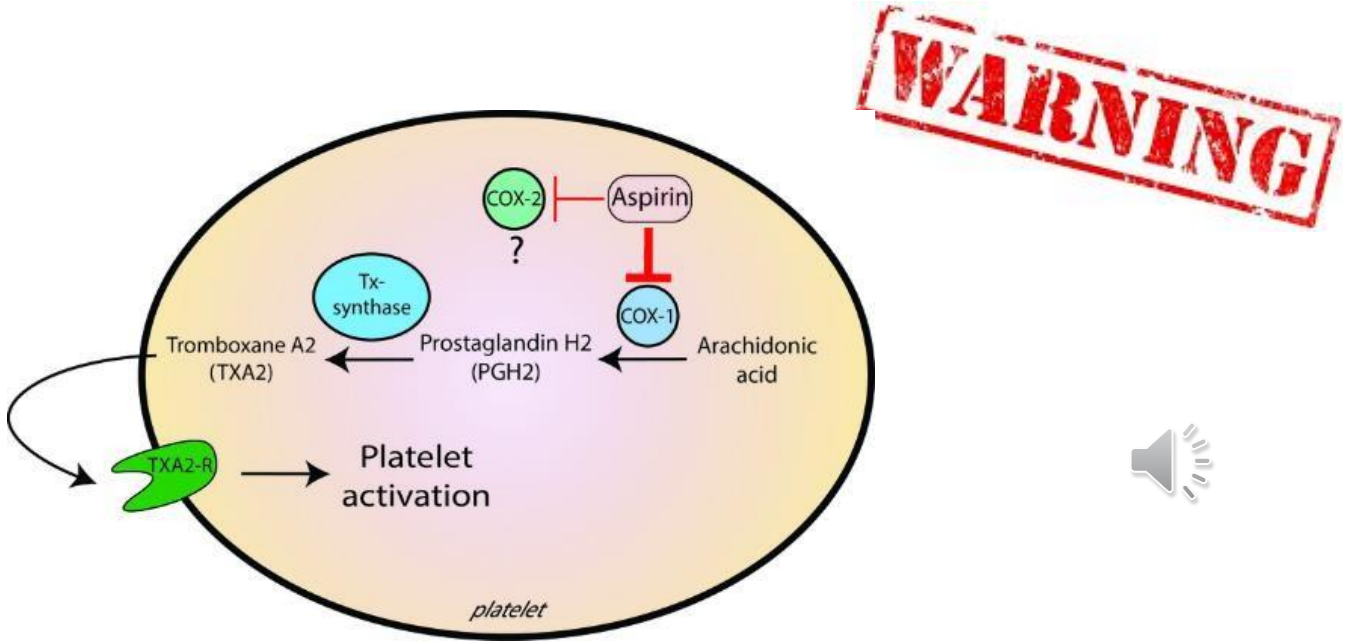
5. Thromboxane Synthesis

1) Ca^{2+} also activates **Phospholipase A_2 (PLA_2)**.
2) PLA_2 cleaves membrane phospholipids to release **arachidonate (arachidonic acid)**.
3) Arachidonate is used to produce prostaglandin intermediates.
4) **Cyclooxygenase (COX)** participates in the pathway that converts arachidonate-derived intermediates into **thromboxane (TXA_2)**.
5) TXA_2 is released by platelets and promotes **platelet aggregation and vasoconstriction**.

NSAID

- Non-steroidal anti-inflammatory drugs inhibit the cyclooxygenase (inhibiting prostaglandin and thromboxane) accounting for their anticoagulant effects.
  - Aspirin also inhibits production of endothelial prostacyclin, which opposes platelet aggregation and is a vasodilator, but, unlike platelets, these endothelial cells regenerate cyclooxygenase within a few hours. Thus, the overall balance between TxA2 and PGI2 can be shifted in favor of the latter.



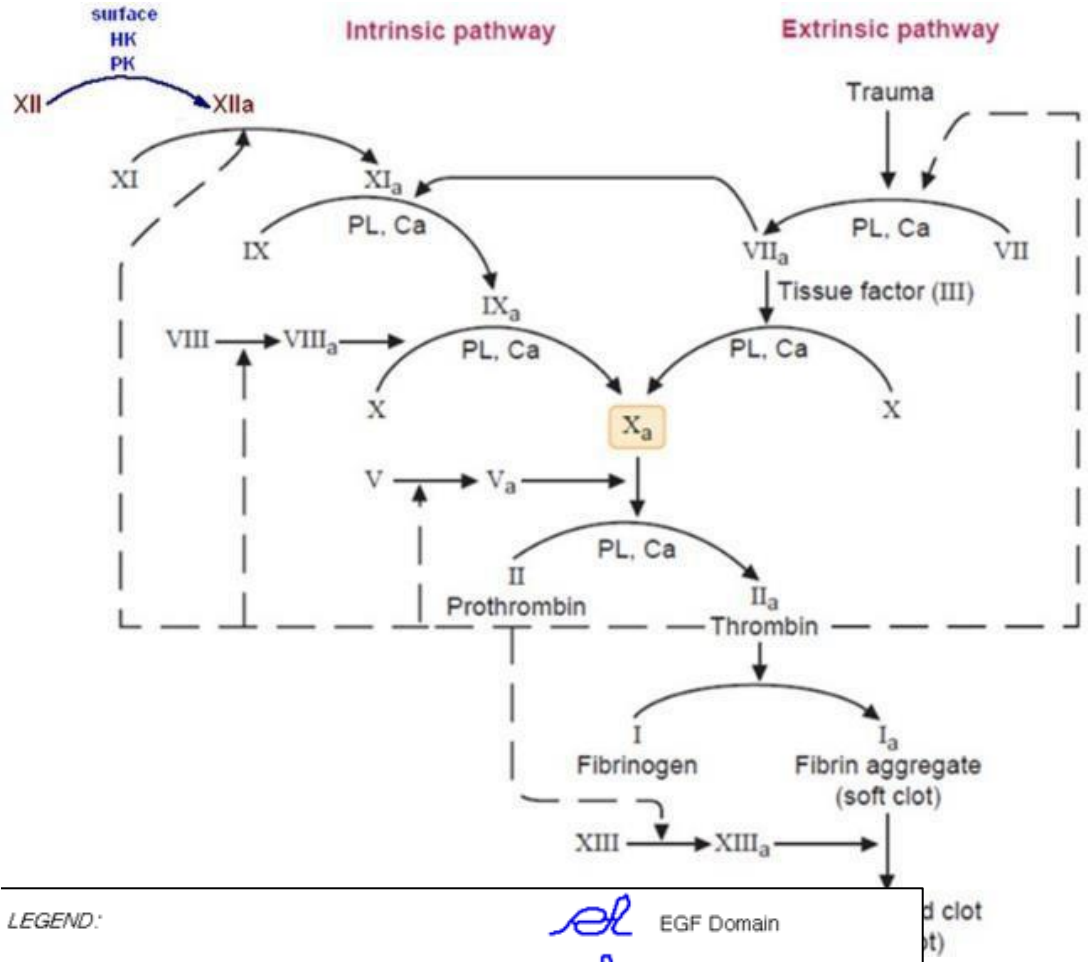


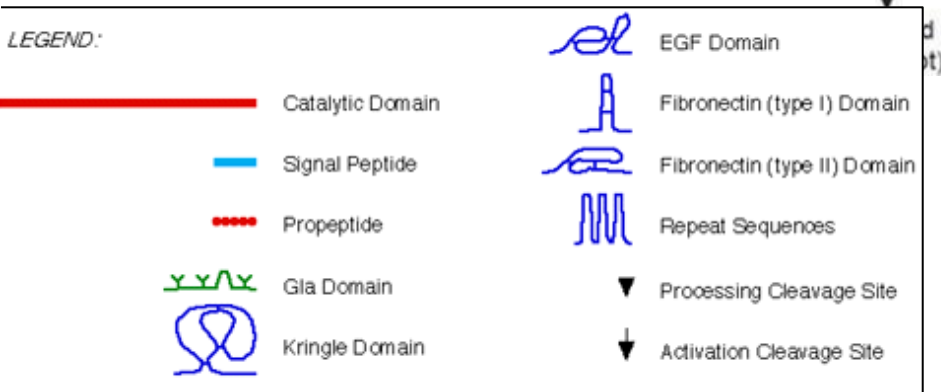
- **Effect on Platelets (Long-term inhibition):** By inhibiting COX inside the platelets, Aspirin drastically reduces the production of prostaglandins and thromboxanes, thereby decreasing blood coagulation for a prolonged period.

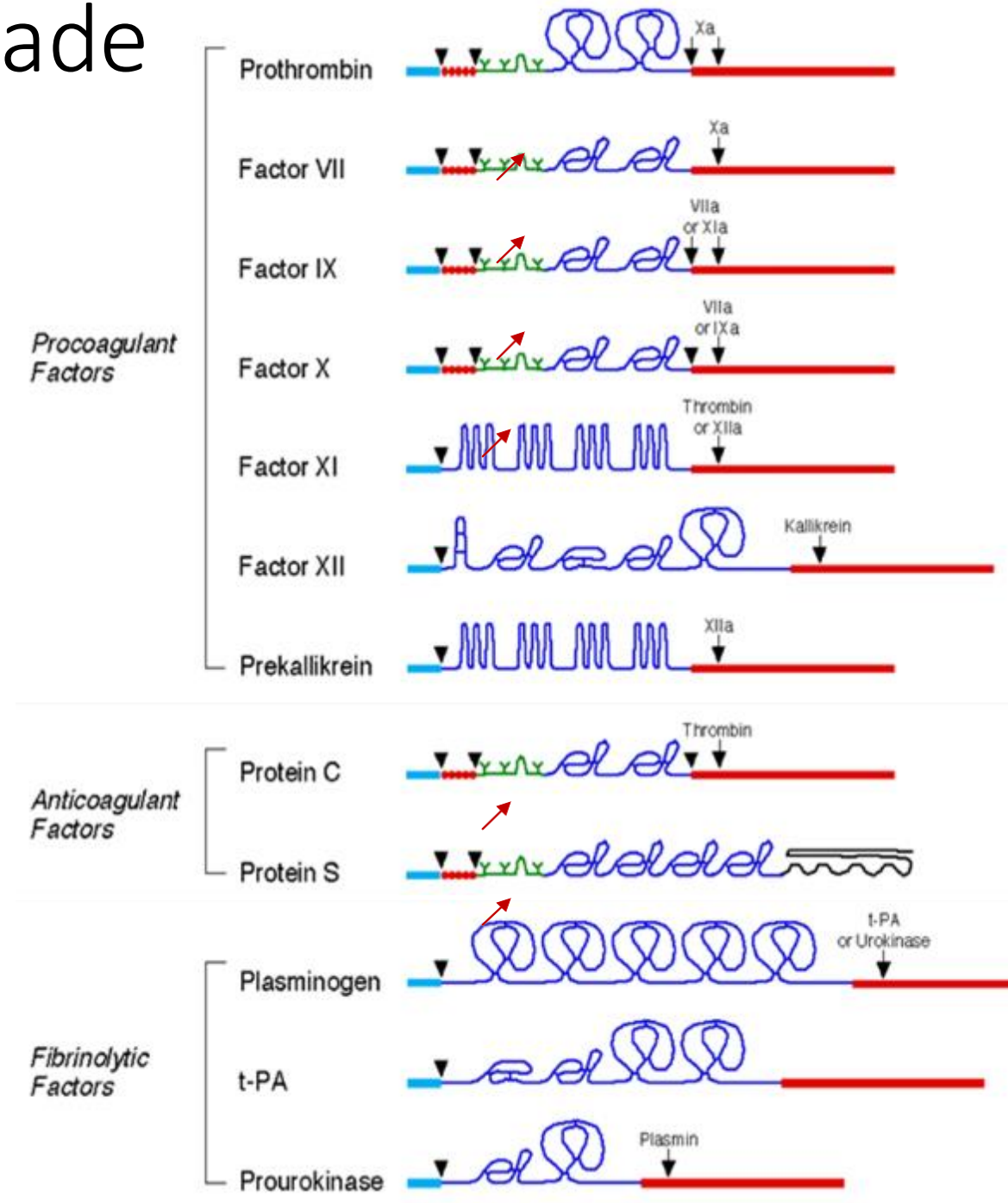
- **Effect on Endothelial Cells (Short-term inhibition):** Aspirin also inhibits COX in endothelial cells, **temporarily** halting their production of Prostacyclin (an innate anticoagulant/vasodilator).

- While inhibiting prostacyclin technically increases coagulation, the endothelial cells regenerate their COX enzyme within a few hours. Thus, the endothelial inhibition is temporary, whereas the platelet inhibition is **long-lasting**, resulting in a net anticoagulant effect.

Coagulation cascade

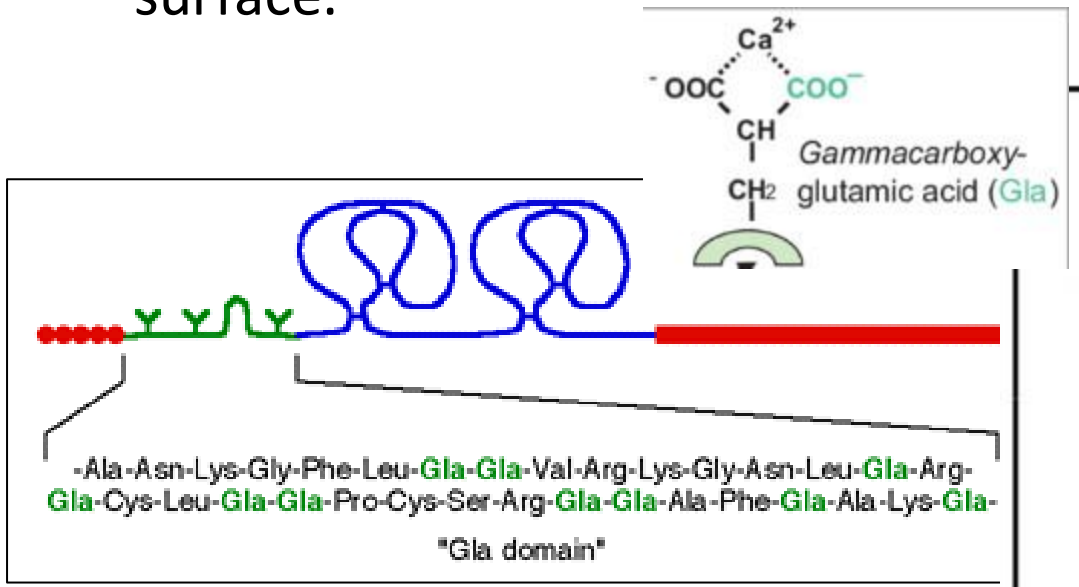


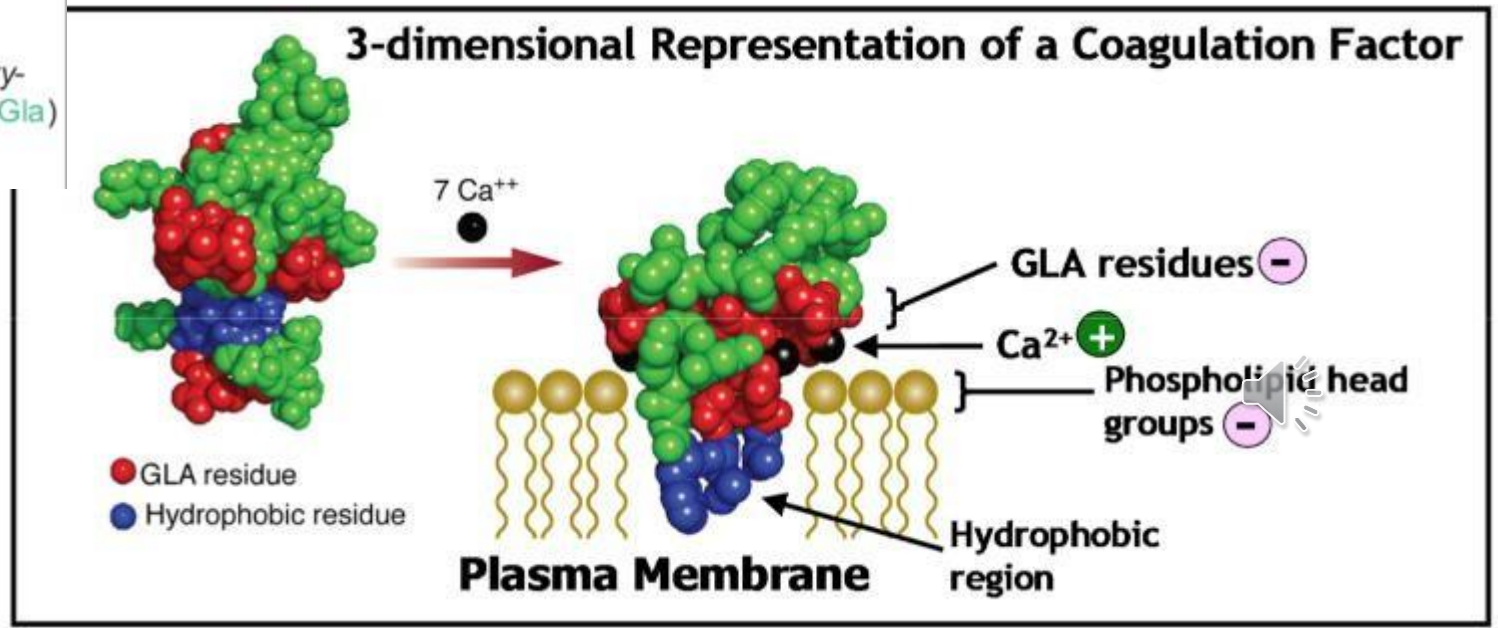




Gla domain

- An ER/Golgi carboxylase binds to prothrombin and factors IX, VII, and X and converts 10≥ glutamate (Glu) residues to γ-carboxyglutamate (Gla), followed by a small (10 a.a.) hydrophobic region.
- The Gla residues bind calcium ions and are necessary for the activity of these coagulation factors and formation of a coordinated complex with the charged platelet surface to localize the complex assembly and thrombin formation to the platelet surface.





- **The Gla Domain:** Present prominently on Factors II, VII, IX, and X. It consists of **multiple glutamic acid residues** grouped together.

- **Role of Negative Charges:** The body adds an extra carboxyl group (COO^-) to the normal glutamate residues on these factors, vastly increasing their negative charge.

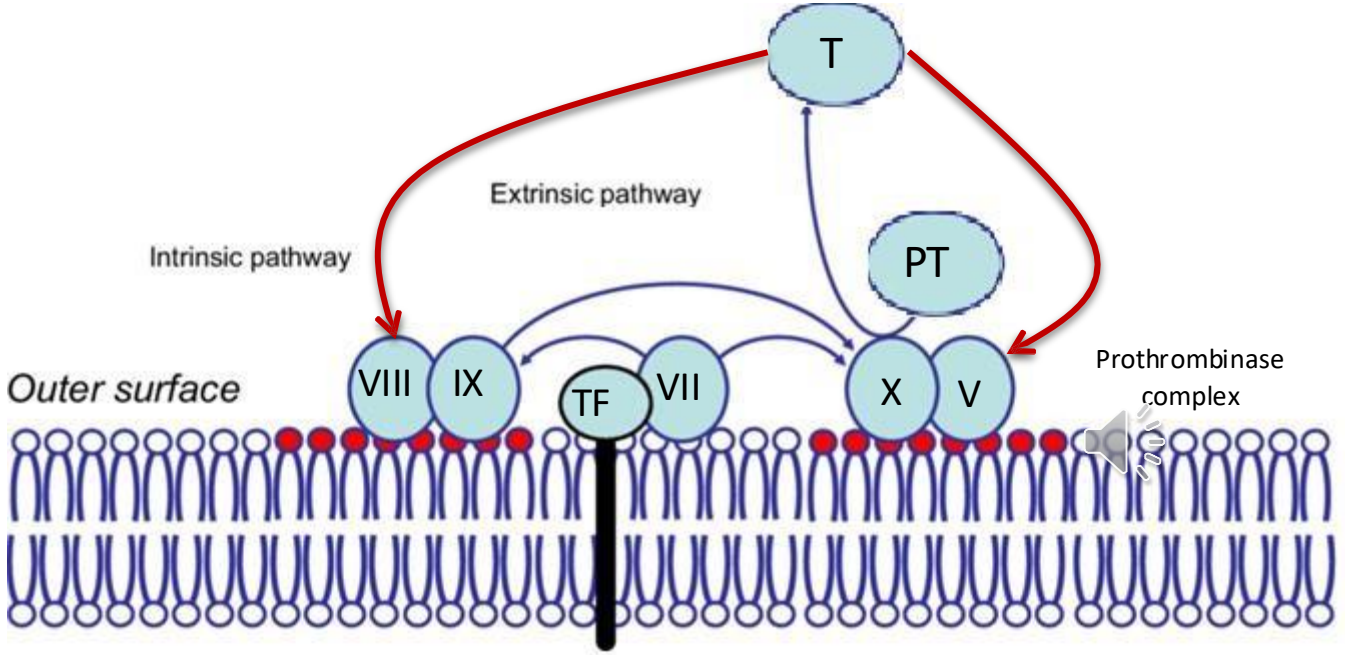
- **Calcium as a Bridge:** These newly formed highly negative Gla domains rely on Calcium (Ca^{2+}) to act as a bridging ion. Calcium binds the negative COO^- of the coagulation factor to the negatively charged phospholipid bilayer of the platelet's surface.

- **Importance of Platelet Surface:** Factors II, VII, IX, and X must bind to the lipid bilayer of the platelet membrane to become functionally active. Calcium is strictly required to facilitate this membrane binding.

The tenase complexes

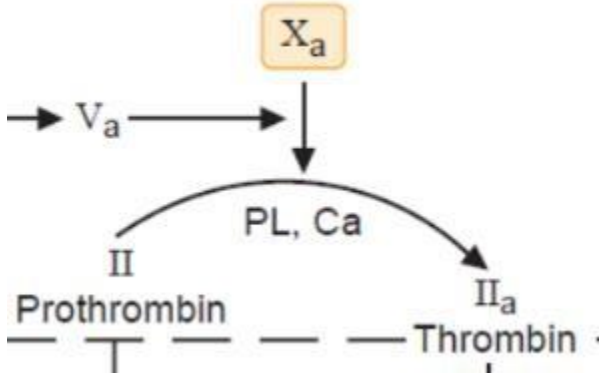
- The activating complexes of factor X are called the "tenase" complexes.
  - The extrinsic tenase complex is made up of tissue factor, factor VIIa, and Ca^{2+}.
  - The intrinsic tenase complex contains the active factor IX (IXa), its cofactor factor VIII (VIIIa), and Ca^{2+}.
    - Tissue factor and factor VIIa also activate factor IX in the intrinsic pathway.
- Prothrombin is also activated by interacting with the platelets' surface.

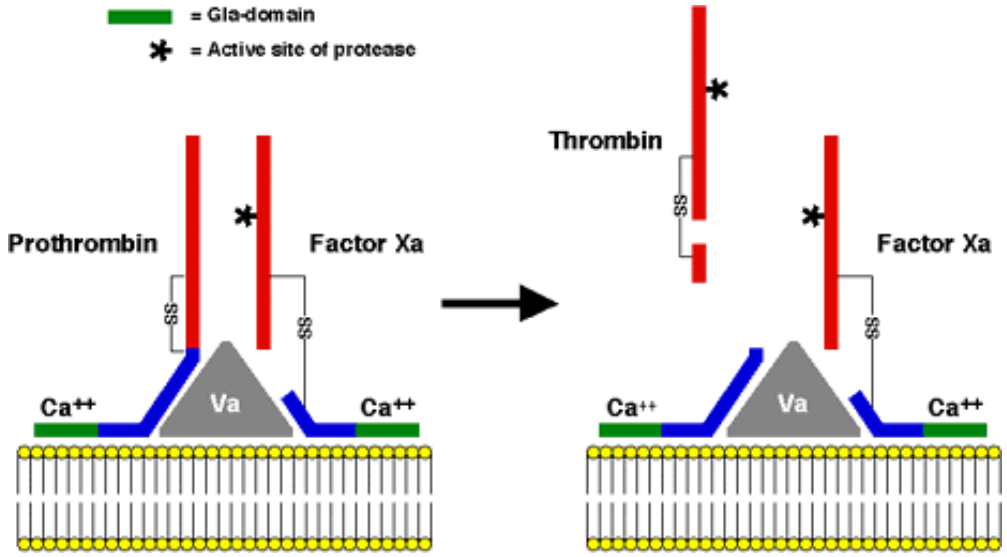
- Va and VIIIa are cofactors that increase the proteolytic efficiency of Xa and IXa, respectively.
  - Both factors V and VIII are activated by thrombin via a feedback mechanism.



Prothrombin activation

- The complex of factor Xa/Va/Ca^{2+} is the “prothrombinase complex”.
- Factor Xa converts prothrombin to thrombin, which is accelerated by Va, platelets (or phospholipids), and calcium ions.
- Binding of calcium alters the conformation the Gla domains of these factors, enabling them to interact with a membrane surface of platelets.
- Aggregated platelets provide the surface upon which prothrombin activation occurs .





The role of vitamin K

- Vitamin K participates in the conversion of Glu to γ-carboxy-Gla by γ-glutamyl carboxylase. Vitamin K donates 2 e- , while the COO- is provided by CO2.
- Vitamin K becomes oxidized and must be regenerated.

1. Vitamin K Function

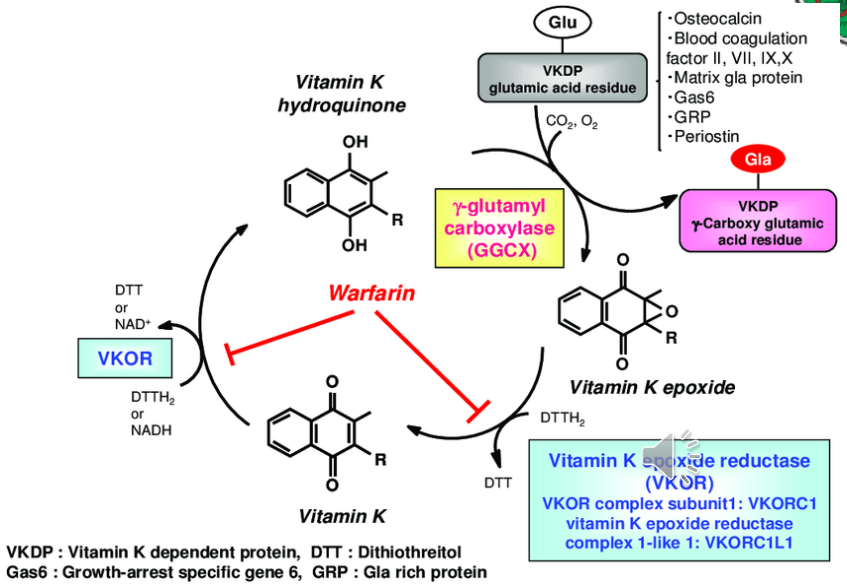
Certain coagulation factors contain glutamate residues that need to be modified by adding an extra **carboxyl group (COO^-)**. This reaction requires **CO_2, O_2, and two electrons**.
Vitamin K supplies the two electrons needed for this process.

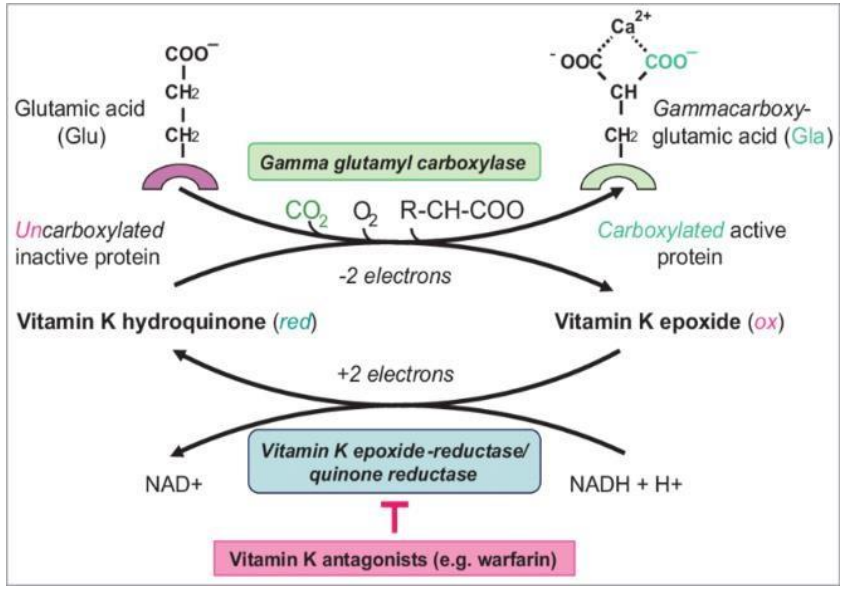
2. Vitamin K Recycling

After donating electrons, Vitamin K becomes **oxidized**. To be used again, it must be converted back to its active, reduced form.
The enzyme **Vitamin K epoxide reductase (VKOR)** carries out this recycling process.

3. Warfarin Mechanism

Warfarin acts as a Vitamin K antagonist. It inhibits Vitamin K epoxide reductase (VKOR).
As a result, oxidized Vitamin K cannot be efficiently recycled into its active form.
Without sufficient active Vitamin K, coagulation factors cannot undergo proper carboxylation. Consequently, these factors cannot acquire the necessary negative charges for normal function, impairing blood coagulation.





Newborns and vitamin K deficiency

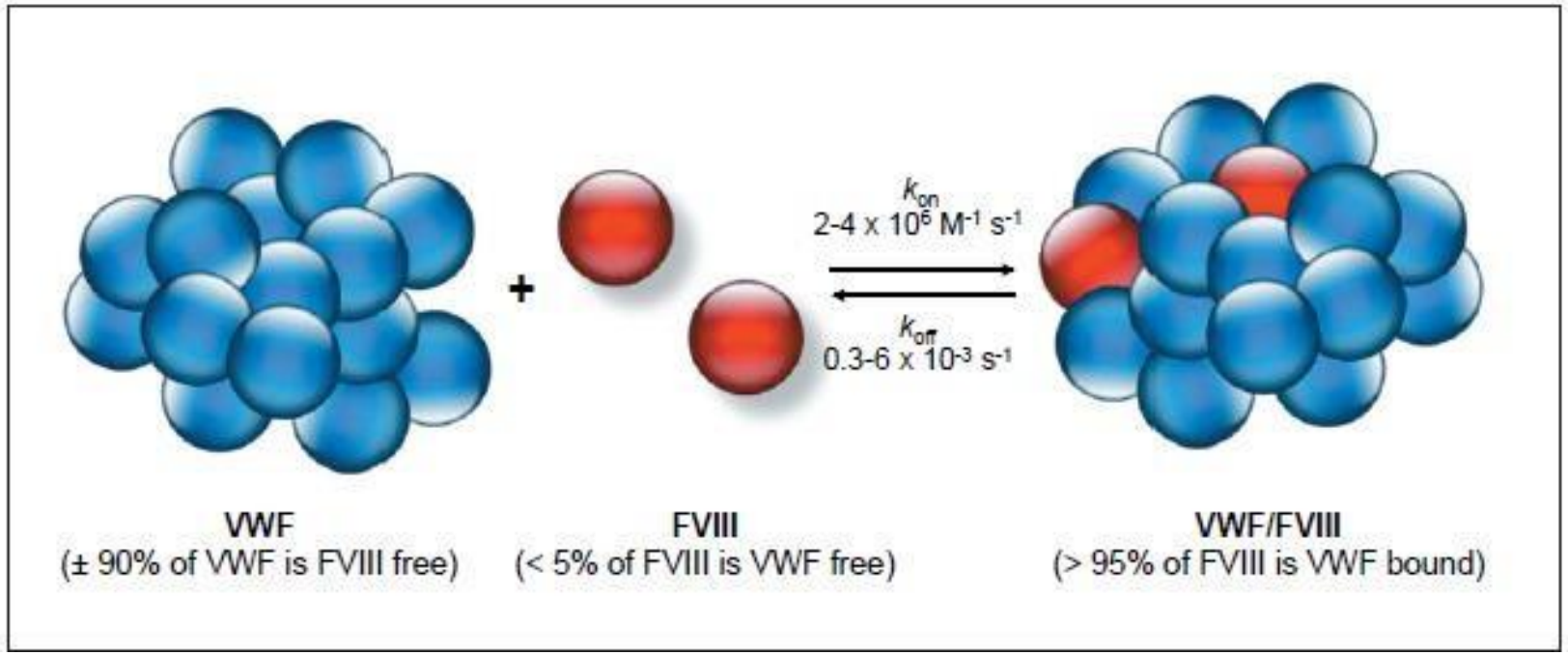
- Newborns are at risk for early vitamin K deficiency bleeding. Why?
  - The placenta is a poor passage channel for fat-soluble compounds, including vitamin K.
  - Neonates are born with an immature liver that impairs coagulation factor synthesis and GLA modifications.
  - Breast milk is a poor source of vitamin K.
  - Intestinal flora, the main source of vitamin K, is not established yet.



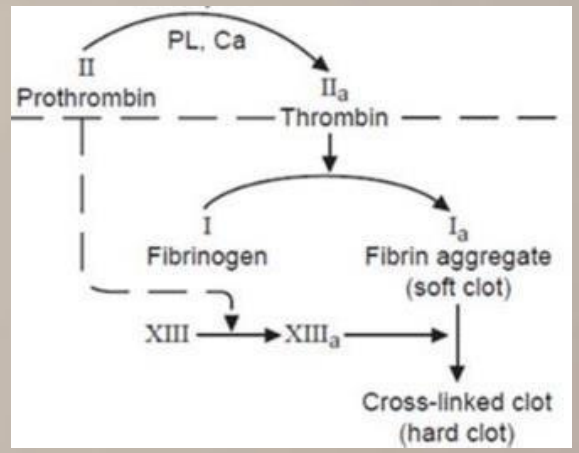


von Willebrand factor deficiency

- The main function of the VWF factor is to stimulate the platelets, but it was found that it also has another function which is the protection of Factor 8.



Endothelial cells



Platelet derived growth factor (PDGF)

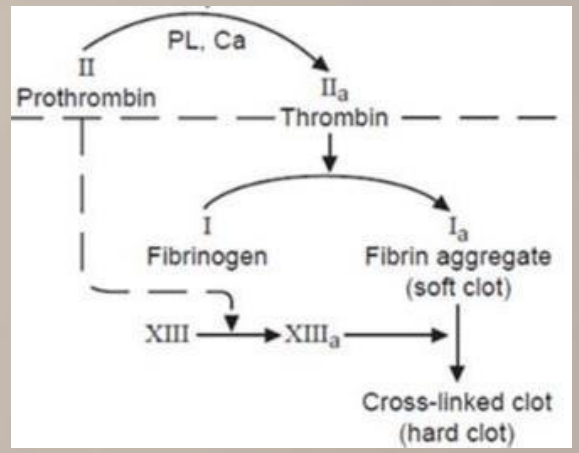
Fibrin forms a mesh around the clot

Vascular endothelial growth factor (VEGF)

Subendothelial tissue

Endothelial cells

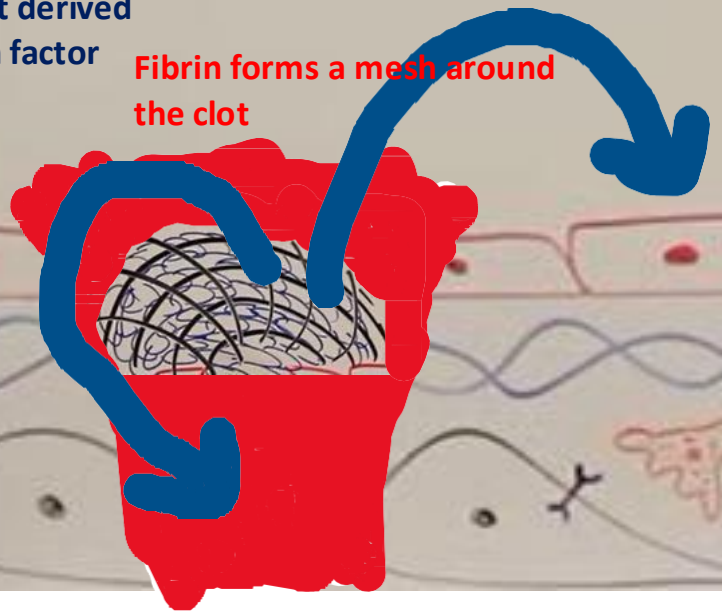
- The last step in clotting is the formation of fibrin mesh around the soft clot. The fibrin results from the polymerization of the ***soluble*** fibrinogen into insoluble ***fibrin*** with the aid of thrombin.
- Thrombin also activates factor XIII which crosslinks the fibrinogen molecules by binding glutamine from one molecule with lysine in another.
- The platelet as a whole produces the Platelet derived growth factor (PDGF) which induces the muscle tissue and subendothelial cells to repair and proliferate.
- The Vascular endothelial growth factor (VEGF) induces the endothelium to repair and proliferate.



Platelet derived growth factor (PDGF)

Fibrin forms a mesh around the clot

Vascular endothelial growth factor (VEGF)



Subendothelial tissue

1. Fibrinogen and Soft Clot Formation

- **Fibrinogen** is a soluble protein in the blood.
- **Thrombin cleaves fibrinopeptides** from fibrinogen through targeted proteolysis.
- This exposes binding sites that allow fibrin molecules to attach to one another.
- The fibrin molecules join together through electrostatic interactions, forming long, insoluble **fibrin threads**.
- These threads create a mesh called the **soft clot**.

2. Hard Clot Formation – Role of Factor XIII

- Thrombin activates **Factor XIII → Factor XIIIa**.
- Factor XIIIa strengthens the fibrin mesh by forming covalent bonds between neighboring fibrin molecules.
- It links a **glutamine residue** on one fibrin molecule to a **lysine residue** on another.
- These cross-links make the clot stronger and more stable, converting the soft clot into a **hard, cross-linked clot**.

3. Tissue Repair Phase

- As the clot forms, platelets release growth factors that help repair the damaged blood vessel.

❑ **PDGF (Platelet-Derived Growth Factor):**

- Targets subendothelial tissue and muscle cells.
- Stimulates these cells to divide and proliferate, helping repair structural damage.

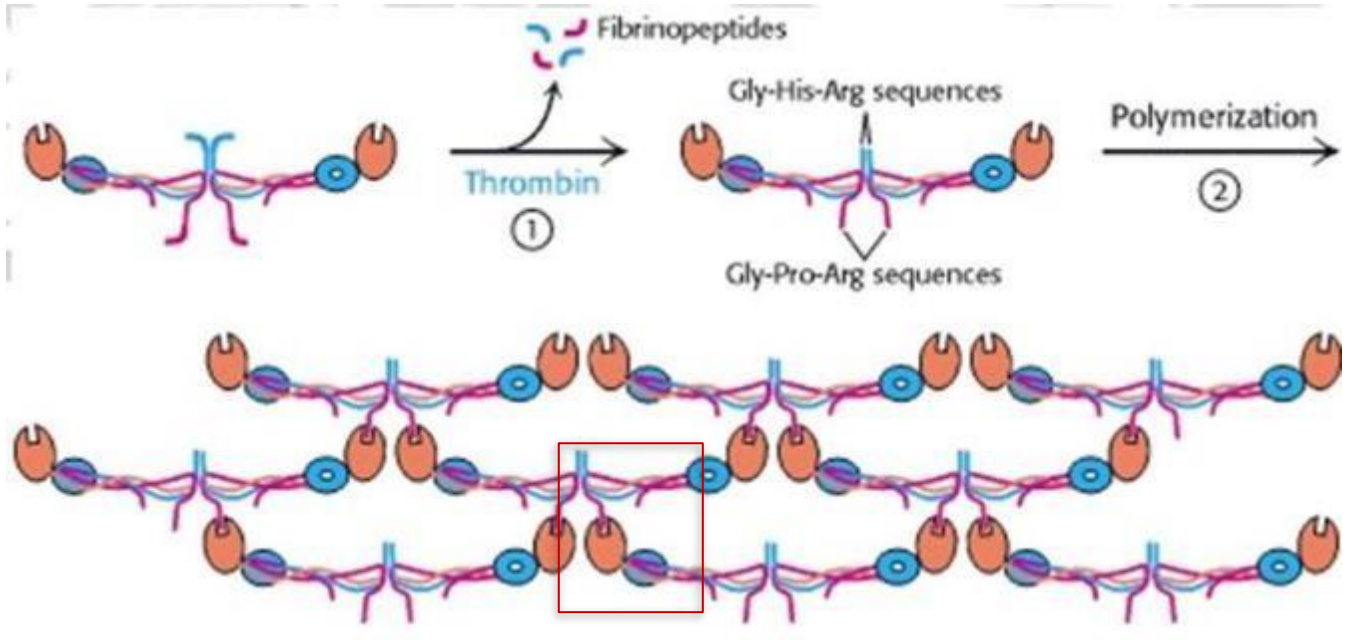
❑ **VEGF (Vascular Endothelial Growth Factor):**

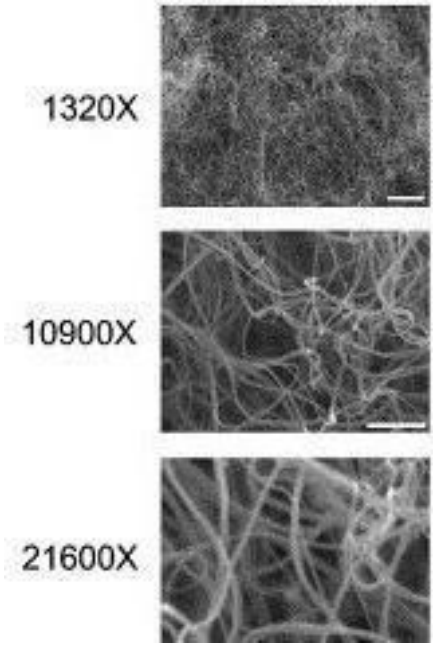
- Targets endothelial cells.
- Stimulates their proliferation to regenerate the damaged blood vessel lining.

Formation of a *soft* fibrin clot



- Thrombin cleaves fibrinogen releasing fibrinopeptides.
  - Fibrinogen is a two triple-stranded helical protein held together by disulfide bonds.
- Fibrin molecules create electrostatic attractions among each other facilitating the aggregation of the monomers into a gel consisting of long polymers.
- The clot resulting from aggregation of fibrin monomers is referred to as the "soft clot“.

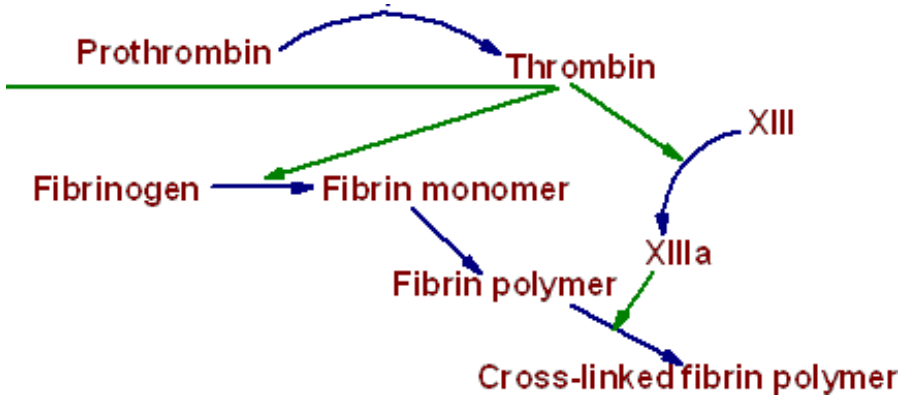


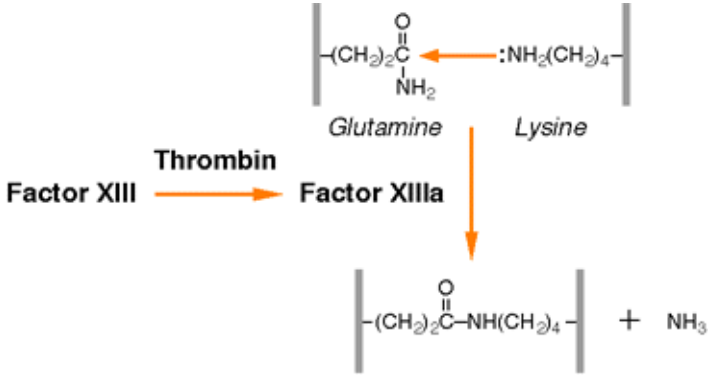


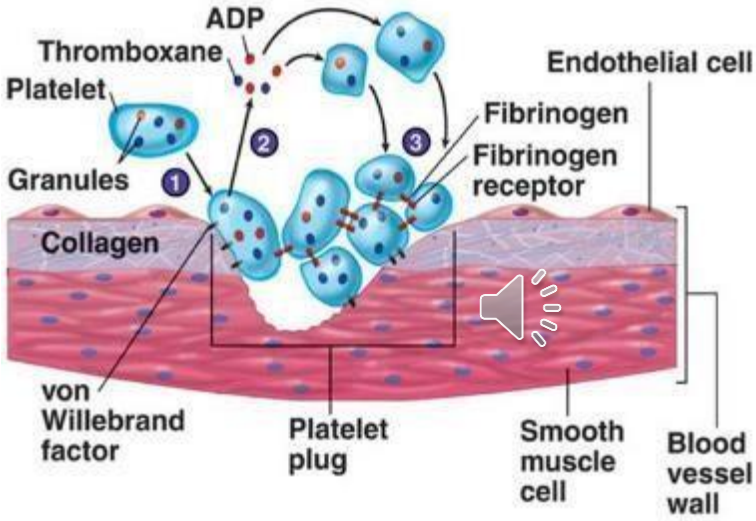
The formation of a hard clot by factor XIII



- Factor XIII is a transglutaminase that is activated by thrombin.
- Factor XIIIa catalyzes a transglutamination reaction that causes a covalent cross-linking reaction between a glutamine of one fibrin monomer to a lysine of an adjacent fibrin monomer.
  - It also cross-links the fibrin clot to adhesive proteins on the endothelial tissue and to the platelet surfaces strengthening the platelet plug.
  - The cross-links strengthen the fibrin mass, forming the "hard clot“
- Notice the difference between the Thrombin’s function and factor XIII function.







Major functions of the coagulation cascade

- Formation of a mesh around the clot
- Activating the platelets through Thrombin.
- Later, they aid in anti clotting and clot resolution.

❑ **Thrombin's Dual Impact ::**

1. Promotes clot formation :

- **Fibrin formation:** Thrombin converts fibrinogen into fibrin, forming the clot's meshwork.
- **Platelet recruitment:** Thrombin also acts as a powerful signaling molecule that activates and recruits more platelets, amplifying clot formation.

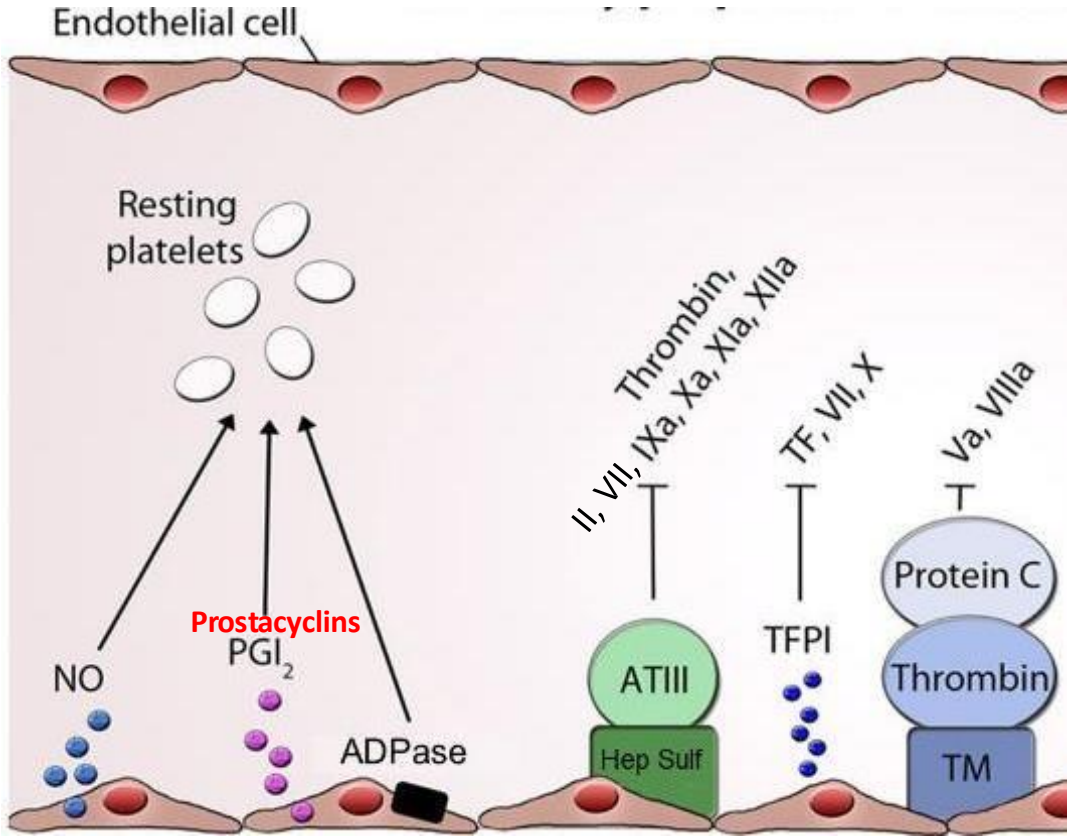
2. Shifts toward clot removal

- Once coagulation has successfully stopped the bleeding, the body increasingly activates **anti-clotting mechanisms** to prevent excessive clot formation.
- Eventually, **fibrinolysis** breaks down the clot when it is no longer needed.

Anti-clotting factors



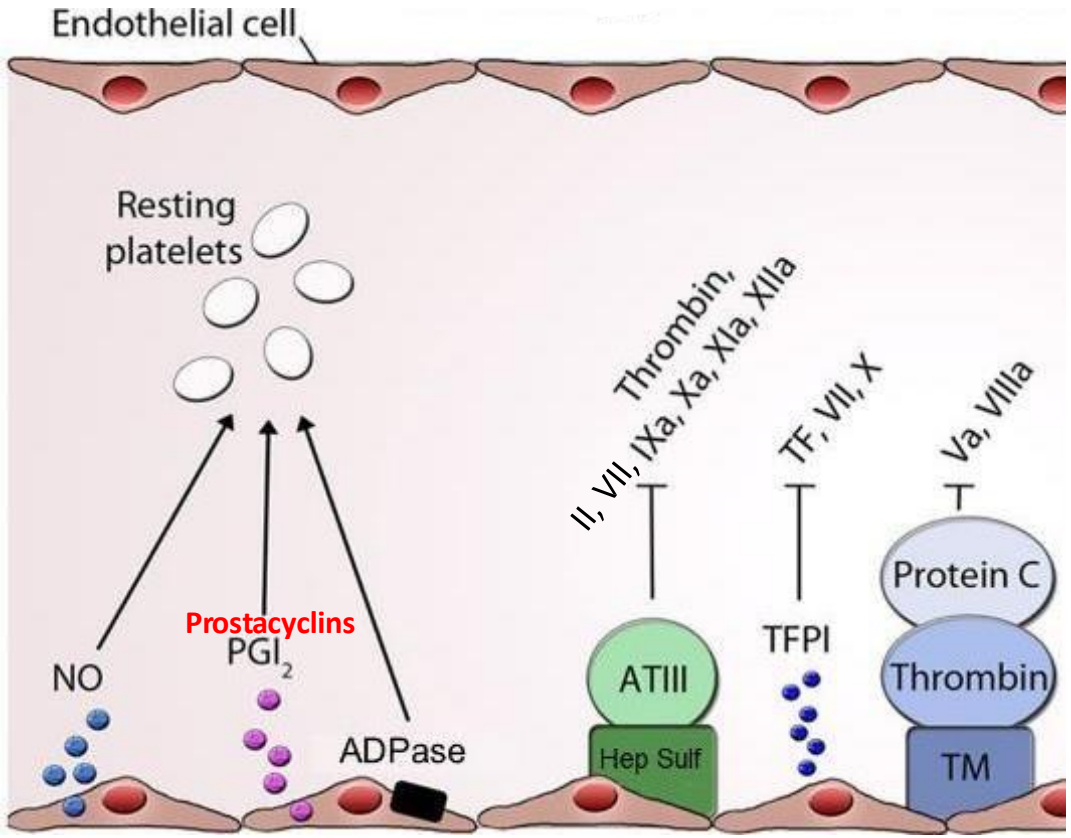
Role of endothelial cells in coagulation



- ECs release NO, prostacyclin (PGI2), and ADPase, which inhibit platelet adhesion and aggregation.
- Membrane-bound heparin sulfate binds to antithrombin III (ATIII) inactivating several coagulation factors.
- ECs express tissue factor pathway inhibitor (TFPI), which inhibits tissue factor (TF) and, consequently, factors VII, IX, and X.
- Thrombomodulin (TM) binds thrombin activating protein C, which degrades factors Va and VIIIa.

Neubauer and Zieger. Endothelial cells and coagulation. Cell and Tissue Research (2021)

Other anticoagulants



Other anticoagulants:

- **Warfarin:** Antagonist for vitamin K.
- **Aspirin:** Inhibitor of COX thus inhibiting the thromboxanes and reducing the platelet activity.
- **Plavix (Clopidogrel):** ADP receptor inhibitor.
- Ca+2 Chelators such as **EDTA (not used for humans, only for phlebotomy tubes)**

- ECs release NO, prostacyclin (PGI2), and ADPase, which inhibit platelet adhesion and aggregation.
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- Thrombomodulin (TM) binds thrombin activating protein C, which degrades factors Va and VIIIa.

Neubauer and Zieger. Endothelial cells and coagulation. Cell and Tissue Research (2021)

Endogenous Anti-Clotting Mechanisms ::

- The body must prevent clotting from spreading uncontrollably and blocking blood vessels, such as those supplying the heart or brain. It uses several natural mechanisms:

1. Nitric Oxide (NO) and Prostacyclin (PGI_2)

- Produced by **endothelial cells** lining blood vessels.
- Cause **vasodilation** and inhibit platelet activation and aggregation.
- This helps prevent unnecessary clot formation inside intact vessels.

2. ADPase

- Breaks down **ADP** in the blood.
- ADP normally recruits and activates platelets.
- Destroying ADP removes an important signal for platelet aggregation.

3. Heparan Sulfate and Antithrombin III

- **Heparan sulfate** enhances the activity of Antithrombin III.
- **Antithrombin III** inhibits several activated coagulation factors, especially thrombin (IIa) and factor Xa, and also IXa, XIa, and XIIa.
- This slows down the coagulation cascade.

4. Tissue Factor Pathway Inhibitor (TFPI)

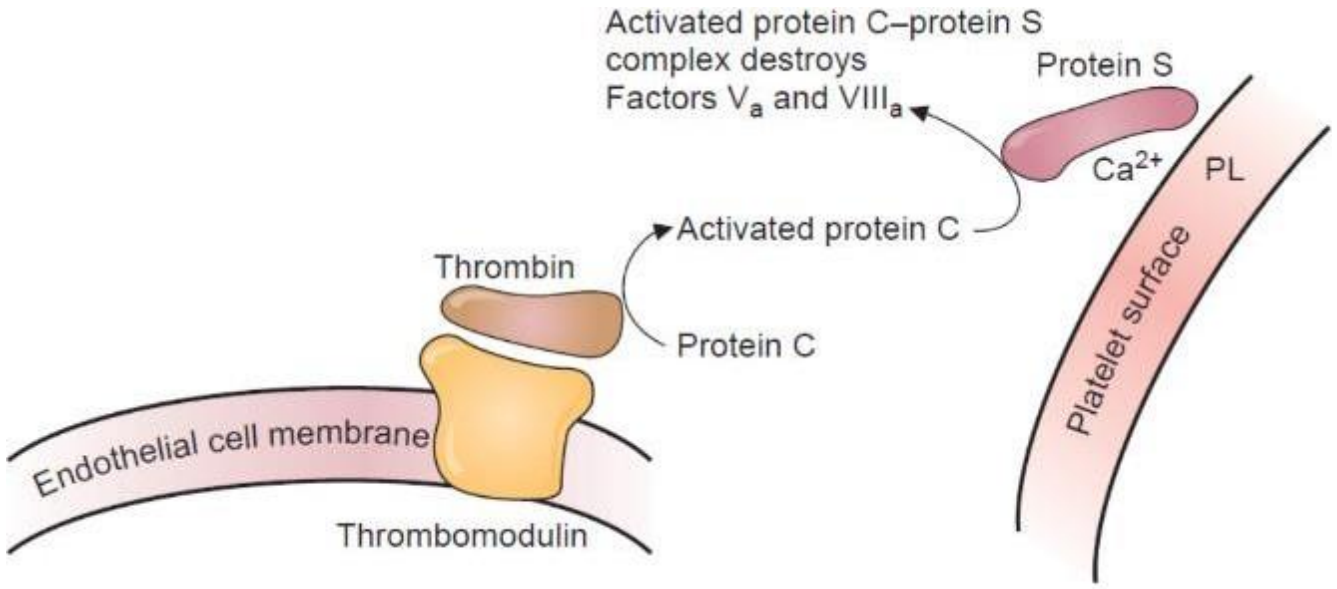
- Specifically inhibits the **tissue factor–Factor VIIa complex** and Factor Xa.
- This limits the **extrinsic pathway** and prevents excessive coagulation.

❑ Thrombin's Negative Feedback Mechanism

- Thrombin has a second role: it can help **switch off further coagulation**.
- Excess thrombin binds to **thrombomodulin**, a receptor on endothelial cells.
- This changes thrombin's function, allowing it to activate **Protein C**.
- Activated Protein C, with Protein S as a cofactor, inactivates **Factors Va and VIIIa**.
- Without these cofactors (Factors Va and VIIIa) coagulation slows down and the formation of additional thrombin decreases.

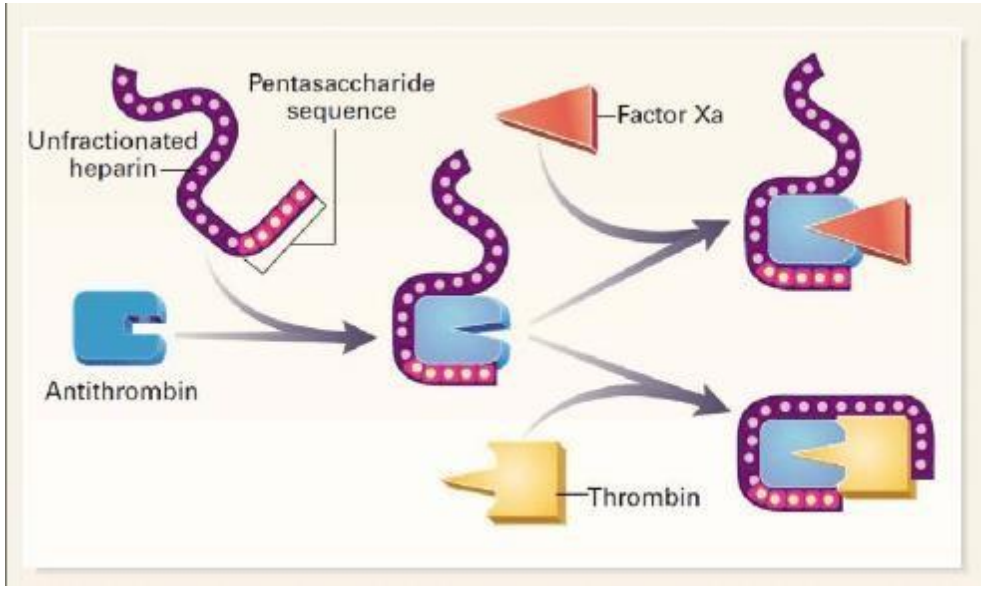
Protein C and protein S

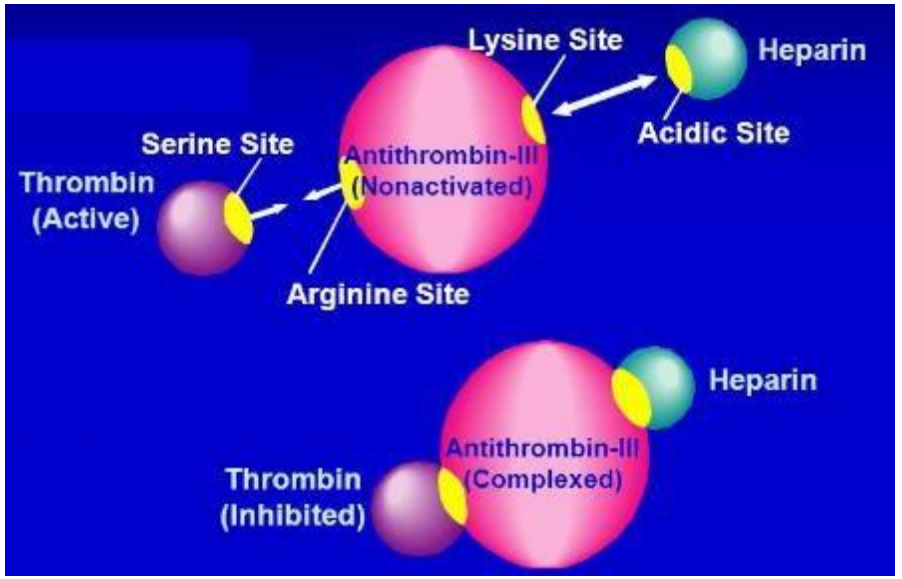
- Thrombin binds thrombomodulin on the surface of endothelial cells.
- Thrombin can then activate protein C, which forms a complex with protein S, both of which are vitamin K-dependent cofactors.
- The complex degrades factors V and VIII.



Antithrombin III

- Antithrombin III is a protease inhibitor of thrombin as well as an inhibitor of IXa, Xa, XIa, XIIa, and VIIa when complexed with TF.
- Heparin sulfate, a polysaccharide synthesized by mast cells and present on the surface of endothelial cells, binds to antithrombin III, promoting binding to its substrates.



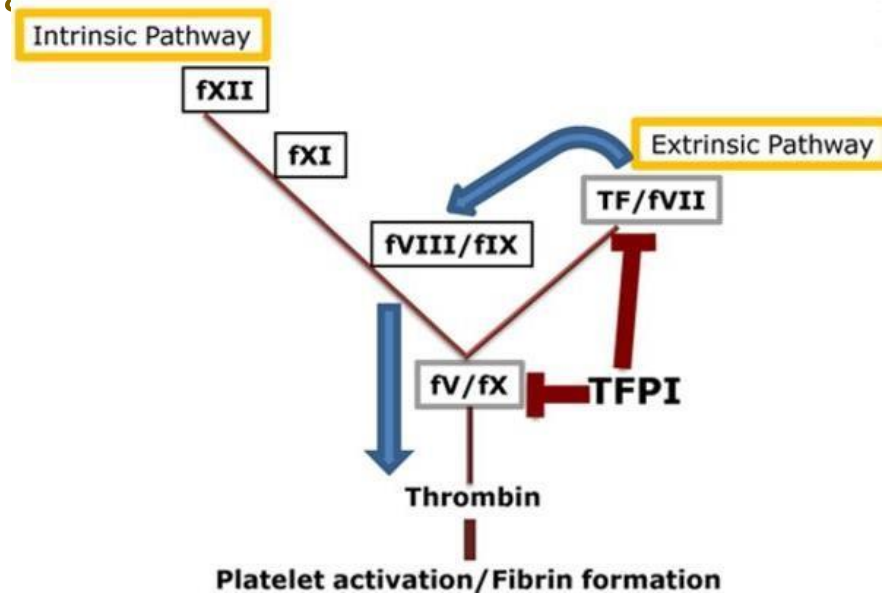


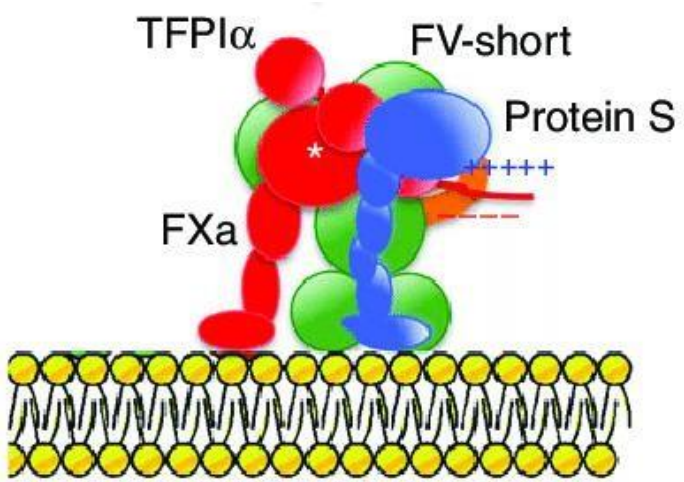


In the clinic, phlebotomy tubes are often treated with heparin to inhibit clot formation.

Tissue Factor pathway inhibitor

- Tissue factor pathway inhibitor (TFPI) is a protein found in plasma lipoproteins and bound to the vascular endothelium.
  - The Xa-TFPI complex then interacts with the TF-VIIa complex and inhibits its activation of factors X and IX.
  - TFPI also inhibits Xa-activated Va resulting in inhibition of the pro-thrombinase complex.
  - Protein S binds to TFPI localizing it to membrane surfaces and enhancing the inhibition of Xa., inhibiting

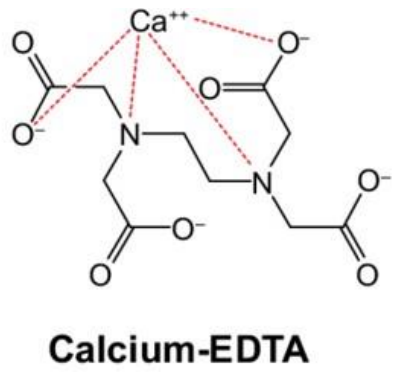




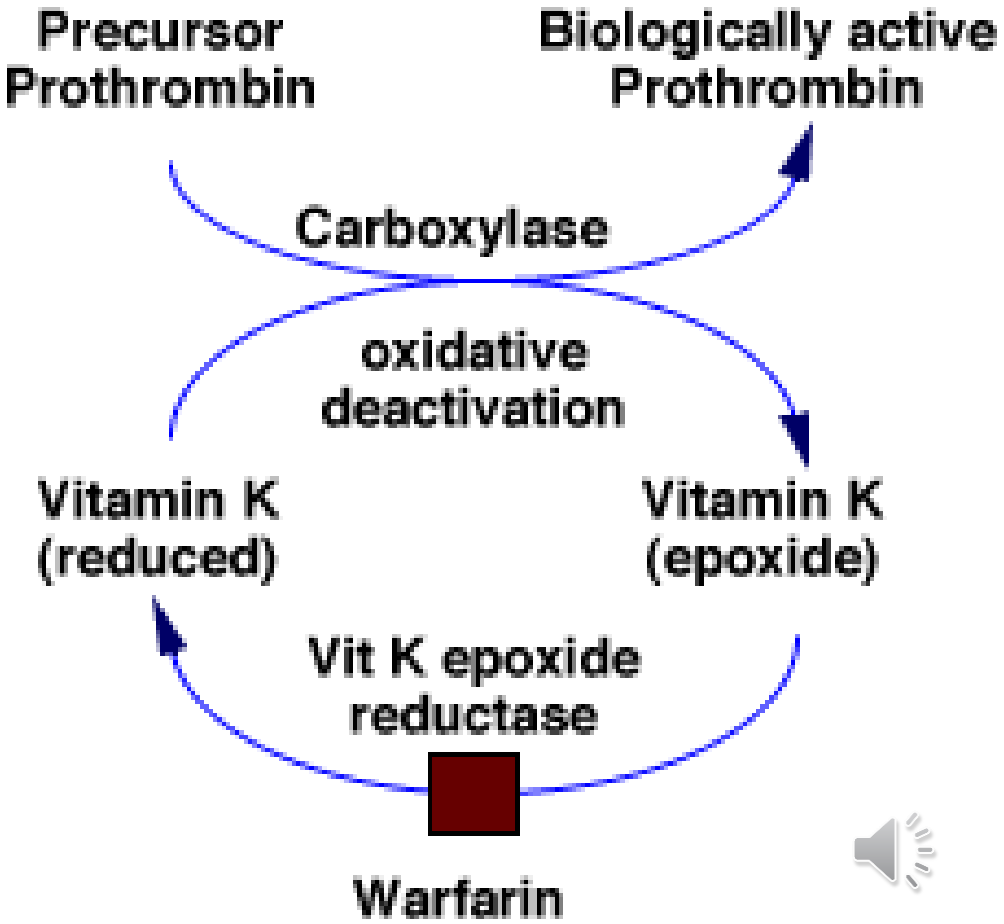
Ca^{2+} chelators and vitamin K antagonists

- Blood clotting can be prevented by addition of Ca^{2+} chelators and vitamin K antagonists such as the drug warfarin, which inhibits the reduction of vitamin K and thereby prevents the synthesis of active prothrombin and factors VII, IX, and X.







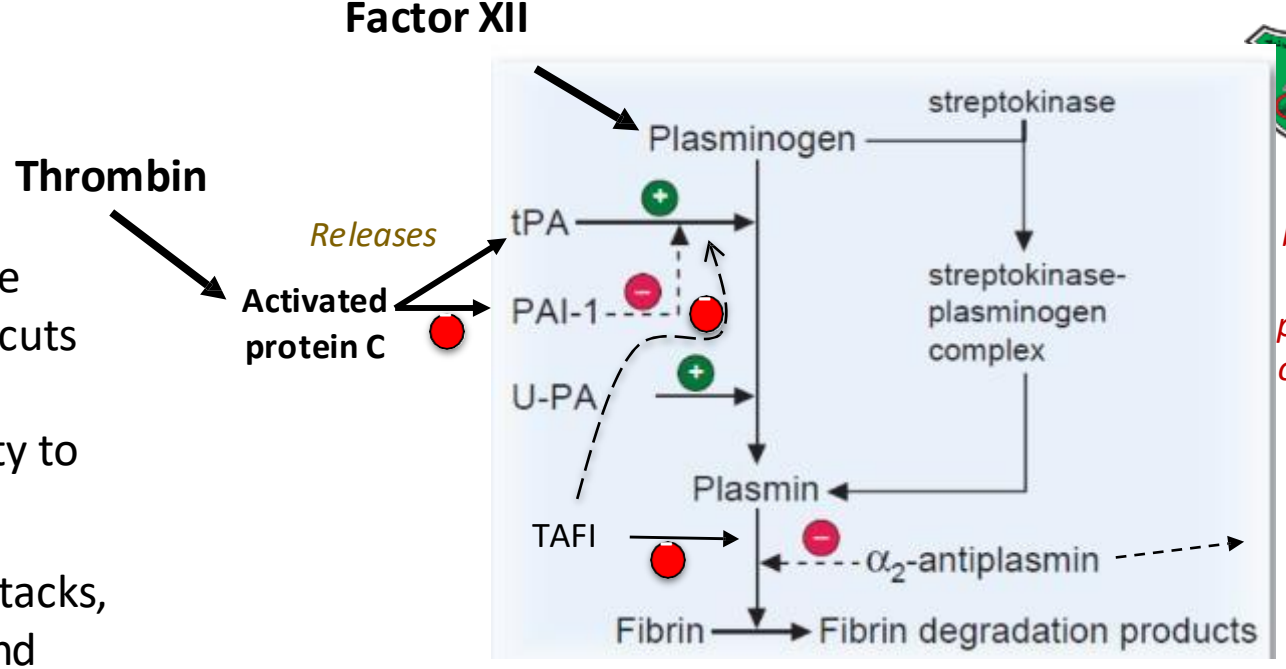


Degradation of the fibrin clot

Clot dissolution

- It is important to prevent clot formation when not needed by anti-clotting factors.
- But if a clot forms , it needs to be dissolved after it finishes its function.

The fibrinolytic system



Factor XII, Fibrin and Thrombin activate Plasminogen through the tissue plasminogen activator tPA. tPA is the main player in the fibrinolysis. Plasmin is like trypsin it cuts proteins at certain sequences, and its activity is highly increased upon binding to fibrin as it puts it in proximity to plasminogen.

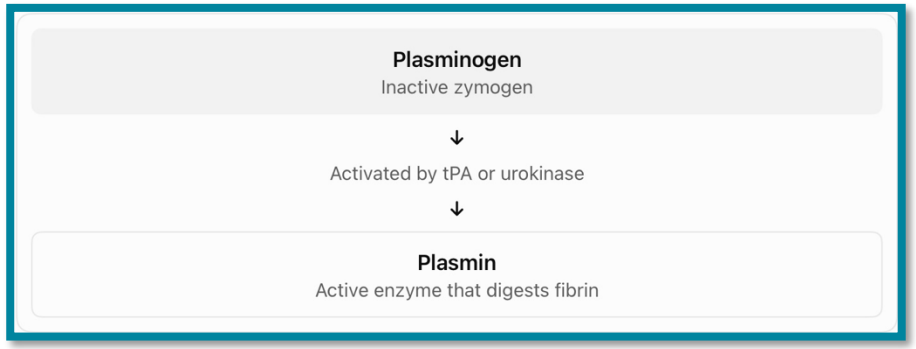
- The most important injectable drugs used for heart attacks, strokes and lung embolisms are called Tenecteplase and Alteplase based on tPA (Both are enzymes).
- Previously streptokinase from the bacteria was used but not anymore.
- There is another activator of plasminogen, but works in the ECM and it activates plasminogen in the ECM to cut the proteins there. This activator is commonly recruited by cancer for invasion. (Urokinase)
- It can nonetheless work on plasminogen everywhere in the body thus increasing the risk of bleeding unlike tPA which is fibrin specific.
- The inhibitors of plasminogen are PAI (by binding to tPA), TAFI (which degrades lysine from fibrin, thus preventing the plasminogen from binding to fibrin), and alpha anti plasmin which inhibits non bound plasmin (very important)
- Some clots are harder to dissolve because they are old or there is no enough plasmin.

Plasmin – The Clot Buster

- Once the damaged tissue has healed, the clot must be removed. This process is called **fibrinolysis**, and it is carried out by **plasmin**, an enzyme that digests the fibrin mesh holding the clot together.

1. Activation of Plasminogen

- Plasmin circulates in the blood in an inactive form called **plasminogen**. It must be activated before it can break down fibrin.



2. Plasminogen Activators

A. Tissue Plasminogen Activator (tPA)

- The main physiological activator of plasminogen.
- Works particularly efficiently when **tPA and plasminogen bind to fibrin**, allowing plasmin to be generated at the clot.
- This helps target clot breakdown to the site where it is needed.

B. Urokinase

- Another activator of plasminogen.
- Functions mainly around cells and the extracellular matrix.
- Some cancer cells exploit this system to facilitate tissue invasion, although urokinase can also contribute to clot breakdown.

3. Fibrinolytic Drugs

- These drugs promote plasmin formation to dissolve dangerous blood clots.
- **Alteplase:** a recombinant form of tPA.
- **Tenecteplase:** a modified form of tPA.
- **Streptokinase:** a bacterial-derived drug that was used historically.
- They may be used in selected emergencies, such as certain heart attacks, ischemic strokes, and pulmonary embolisms, depending on the clinical situation. They are not appropriate for every patient because they can cause serious bleeding.

4. Inhibitors of Fibrinolysis

The body must control plasmin activity so it does not destroy fibrin or other proteins unnecessarily.

Inhibitor	Mechanism
Alpha-2-antiplasmin	Neutralizes free plasmin in the blood, limiting unwanted protein breakdown.
PAI (plasminogen activator inhibitor)	Inhibits tPA and other plasminogen activators, reducing plasmin formation.
TAFI	Removes lysine residues from fibrin, reducing the binding sites needed for tPA and plasminogen.

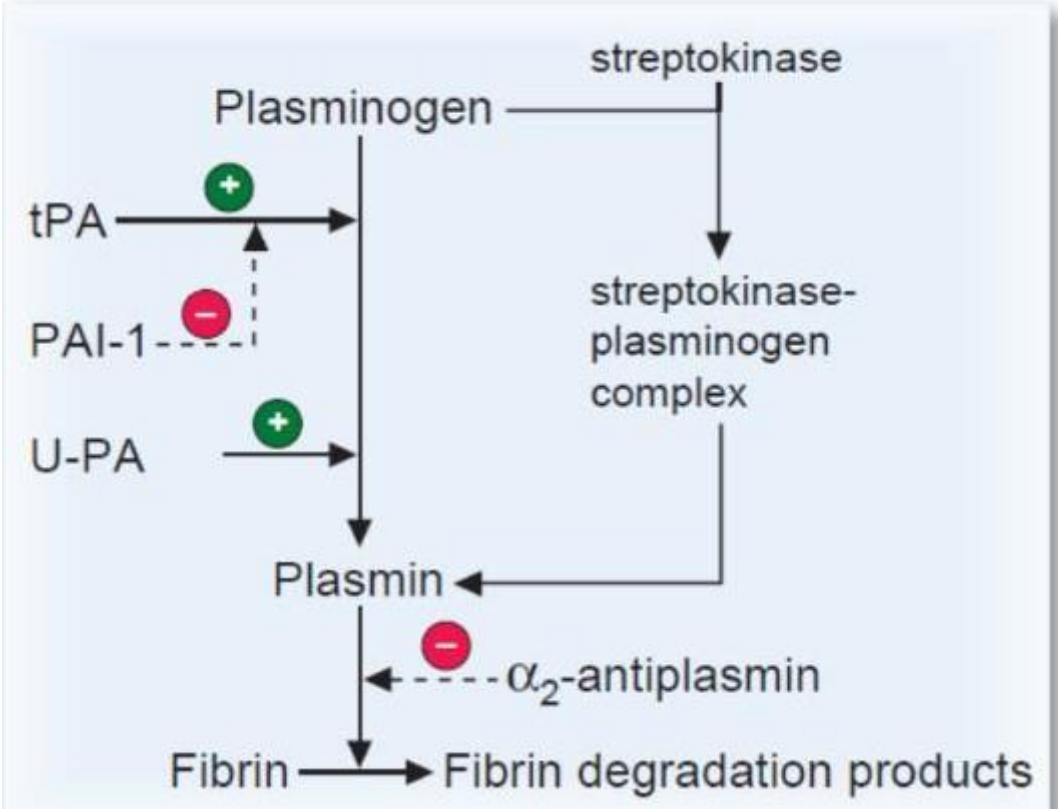
5. Why Are Older Clots More Resistant?

As a clot matures, **Factor XIIIa creates covalent cross-links between fibrin molecules**, making the fibrin network stronger and more stable.

Consequently, older clots can become more resistant to plasmin-mediated breakdown. The degree of resistance depends on clot structure and maturation; the 4–5-hour timing in your notes is a simplified teaching point.

Urokinase

- Urokinase (plasminogen activator) is a protease that is formed from the zymogen pro-urokinase.
- It is a potent plasminogen activator and is used clinically.



- **Note: DO NOT CONFUSE BETWEEN FIBRINOGEN AND PLASMINOGEN**

Roles of thrombin

- Platelet recruitment
- Amplification of the coagulation complex
- Formation of soft clot
  - Proteolytic cleavage of fibrinogen
- Formation of hard clot
  - Activation of factor XIII
- Attenuation of its own activity
  - Activation of protein C
- Other actions
  - Binding to its receptor on the surface of platelets induces vascular remodeling (e.g. angiogenesis) and inflammation.

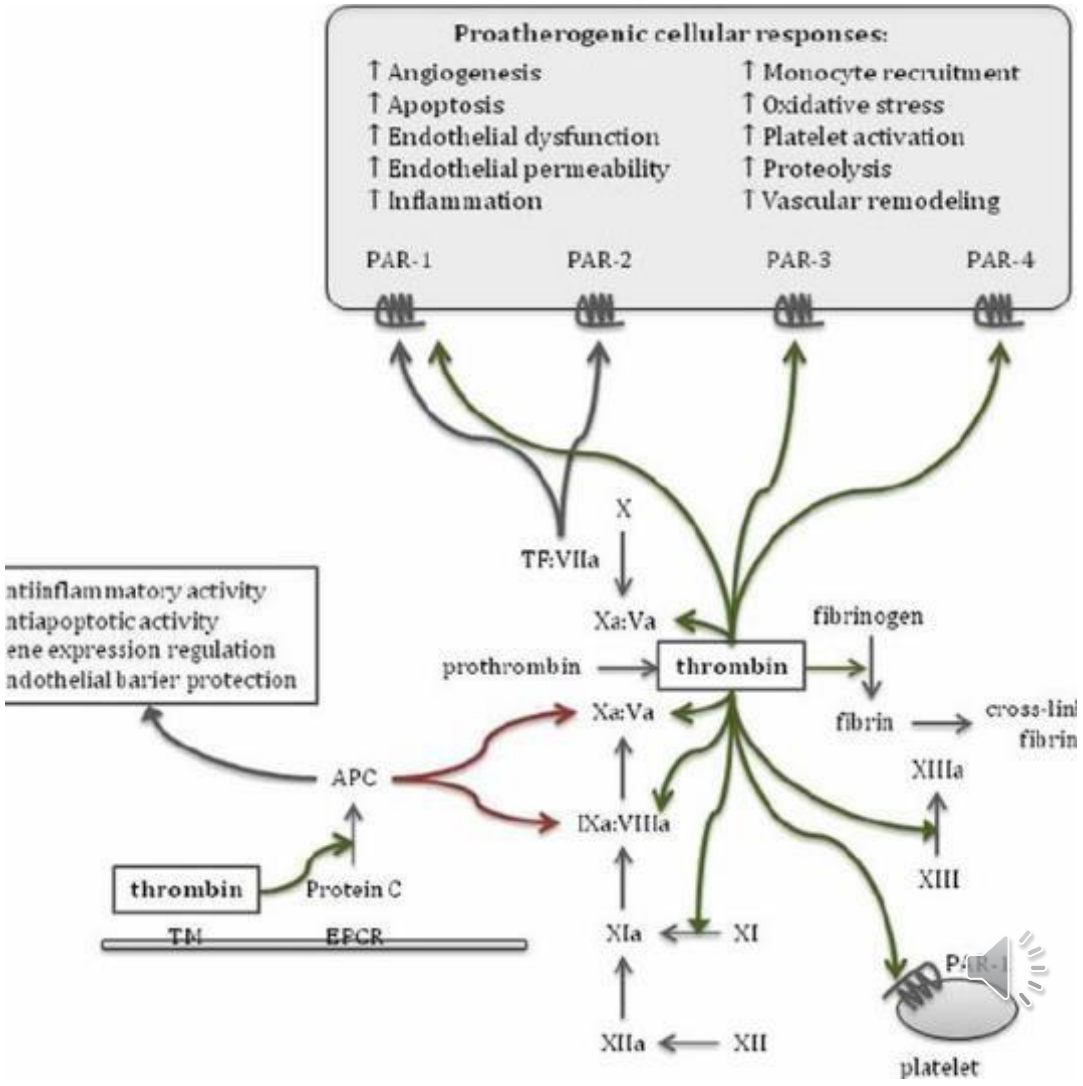


Table 3: illustrates the engagement and detailed explanation of coagulation factors, that aid in the blood coagulation cascade

Factor	Name	Source	Pathway	Description	Function
I	Fib	Liver	Common	Plasma glycoprotein; Molecular Weight (MW)= 340 kilodaltons (kDa)	Adhesive protein which aids in fibrin clot formation.
II	Prothrombin	Liver	Common	Vitamin K-dependent serine protease; MW= 72 kDa	Presence in the activated form and the main enzyme of coagulation
III	Tissue factor	Secrete by the damaged cells and platelets	Extrinsic and Intrinsic	Known as thromboplastin; MW= 37 kDa	Lipoprotein initiator of the extrinsic pathway
IV	Calcium ions	Bone and gut	Entire process	Required for coagulation factors to bind to phospholipid (formerly known as factor IV)	Metal cation which is important in coagulation mechanisms
V	Proaccererin / Labile factor	Liver and platelets	Intrinsic and extrinsic	MW = 330 kDa	Cofactor for the activation of prothrombin to thrombin (prothrombinase complex)
VII	Proconvertin (stable factor)	Liver	Extrinsic	MW = 50 kDa; vitamin K-dependent serine protease	With tissue factor, initiates extrinsic pathway (Factor IX and X)
VIII	Antihemophilic factor A (cofactor)	Platelets and endothelium	Intrinsic	MW = 330 kDa	Cofactor for intrinsic activation of factor X (which it forms tenase complex)
IX	Christmas factor / Antihemophilic factor B (plasma thromboplastin component)	Liver	Intrinsic	MW = 50 kDa; vitamin K-dependent serine protease	Activated form is enzyme for intrinsic activation of factor X (forms tenase complex with factorVIII)
X	Stuart-Prower factor (enzyme)	Liver	Intrinsic and extrinsic	MW = 58.9 kDa; vitamin K-dependent serine protease	Activated form is the enzyme for final the common pathway activation of prothrombin (forms prothrombinase complex with factor V)
XI	Plasma thromboplastin antecedent	Liver	Intrinsic	MW = 160 kDa; serine protease	Activates intrinsic activator of factor IX
XII	Hageman factor	Liver	Intrinsic; (activates plasmin)	MW = 80 kDa; serine protease	Initiates activated partial thromboplastin time (aPTT) based intrinsic pathway; Activates factor XI, VII and prekallikrein
XIII	Fibrin stabilizing factor	Liver	Retards fibrinolysis	MW = 320 kDa; Crosslinks fibrin	Transamidase which cross-links fibrin clot

Molecular components of coagulation

Notes:

1. Names and symbols
2. Pathway
3. Sources
4. Functions
5. Do not worry about MW

Factors: IIa, VIIa, XIa, Xa, XIa, XIIa and Plasmin are all serine proteases.

Summary of the process

- 1- Tissues are injured → Endothelial cells are activated releasing endothelin which activates the smooth muscle cells to contract → Vasoconstriction. Endothelial cells also release vWR. The exposed collagen activates the platelets and the intrinsic pathway. If the wound is deep the extrinsic pathway is activated.
- 2- Platelets attach and become activated releasing compounds to activate further platelets and recruit them to the wound to promote aggregation.
- 3- As a result of activation of the intrinsic and the extrinsic pathways, thrombin converts fibrinogen to fibrin by releasing the firbrinopeptides allowing a mesh to form around the platelets. The mesh is not very strong yet. It is further strengthen by factor 13 which further cross-links it together.
- 4- The clot releases factors that stimulate the endothelial cells and the subendothelial tissue to divide and recover.
- 5- The clot is redissolved by plasmin and other factors that activate the plasmin.

Important anti coagulation drugs and factors mentioned

- 1- Clopidogrel (Plavix) works on the ADP receptor of the platelet, and Aspirin works on COX inside the aspirin → Both work on the platelets.
- 2- Warfarin works on vitamin K affecting the intrinsic and extrinsic pathway causing the clot mesh to be less stable.
- 3- Tenecteplase and Alteplase → Very important medical intervention after heart attacks, embolisms and strokes.
- 4- Heparin, Thrombin, anti Thrombin, ADPase, TFI and factor XII.

Why doesn't the ADP produced from routine ATP use cause platelet activation in the blood?
There are two main reasons:

1. First, ADP largely remains confined inside the cells and does not easily exit into the bloodstream.
2. Second, even if some ADP does leak out, ADPase—present either in the blood or located on the cell surfaces (primarily on the cells)—takes care of it and breaks it down.

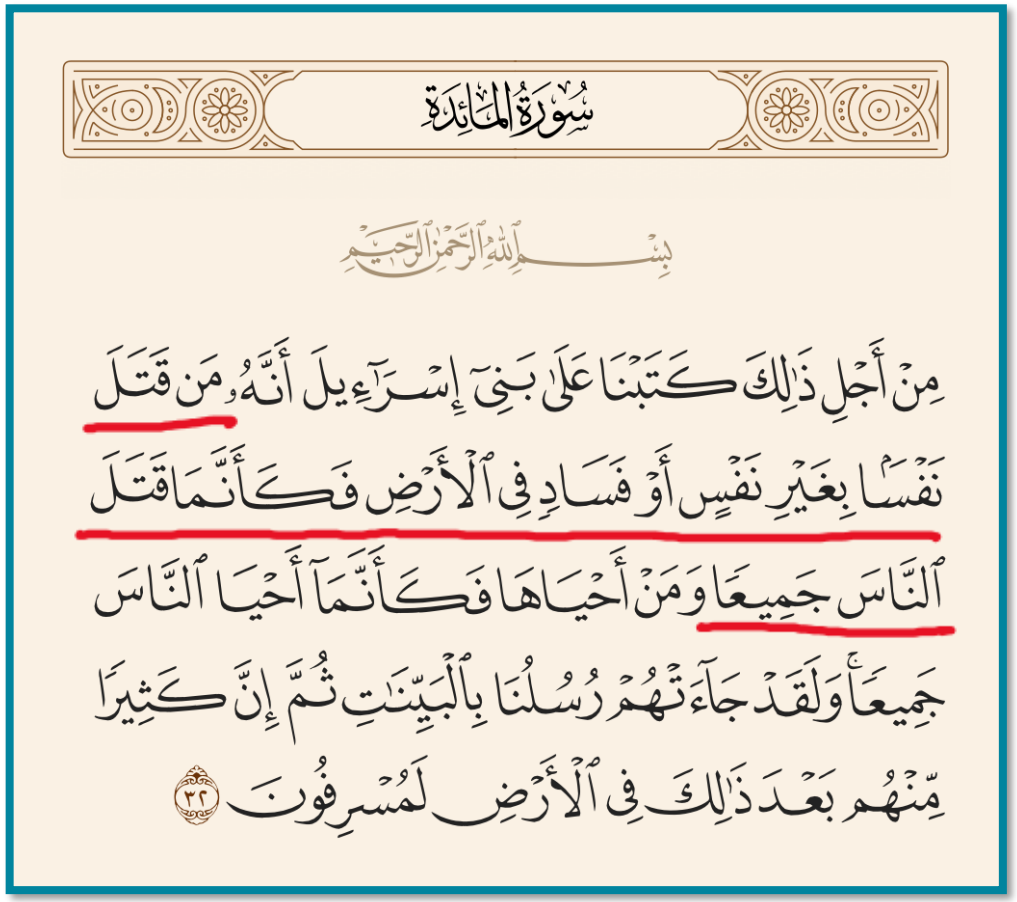
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- مَن قَتَلَ نَفسًا مُعاهَدًا لم يَرِحْ رائِحةَ الجَنَّةِ، وإنَّ ريحَها لَيوجَدُ مِن مَسيرةِ أربَعينَ عامًا.

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Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			