



Blood coagulation

Prof. Mamoun Ahram

Hematopoietic-lymphatic system





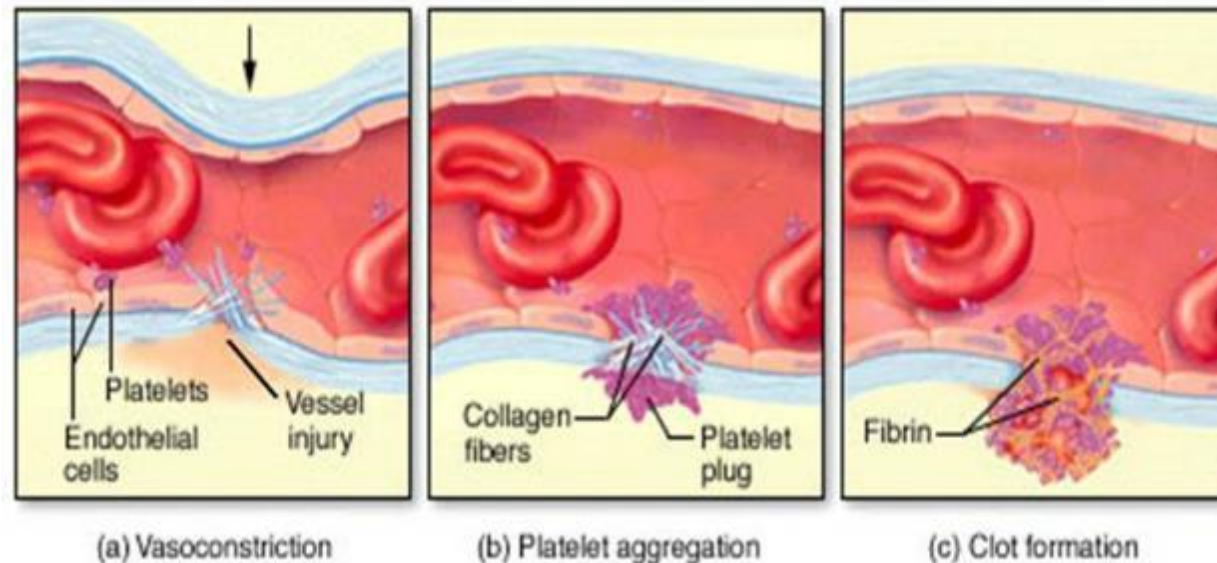
Resources

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

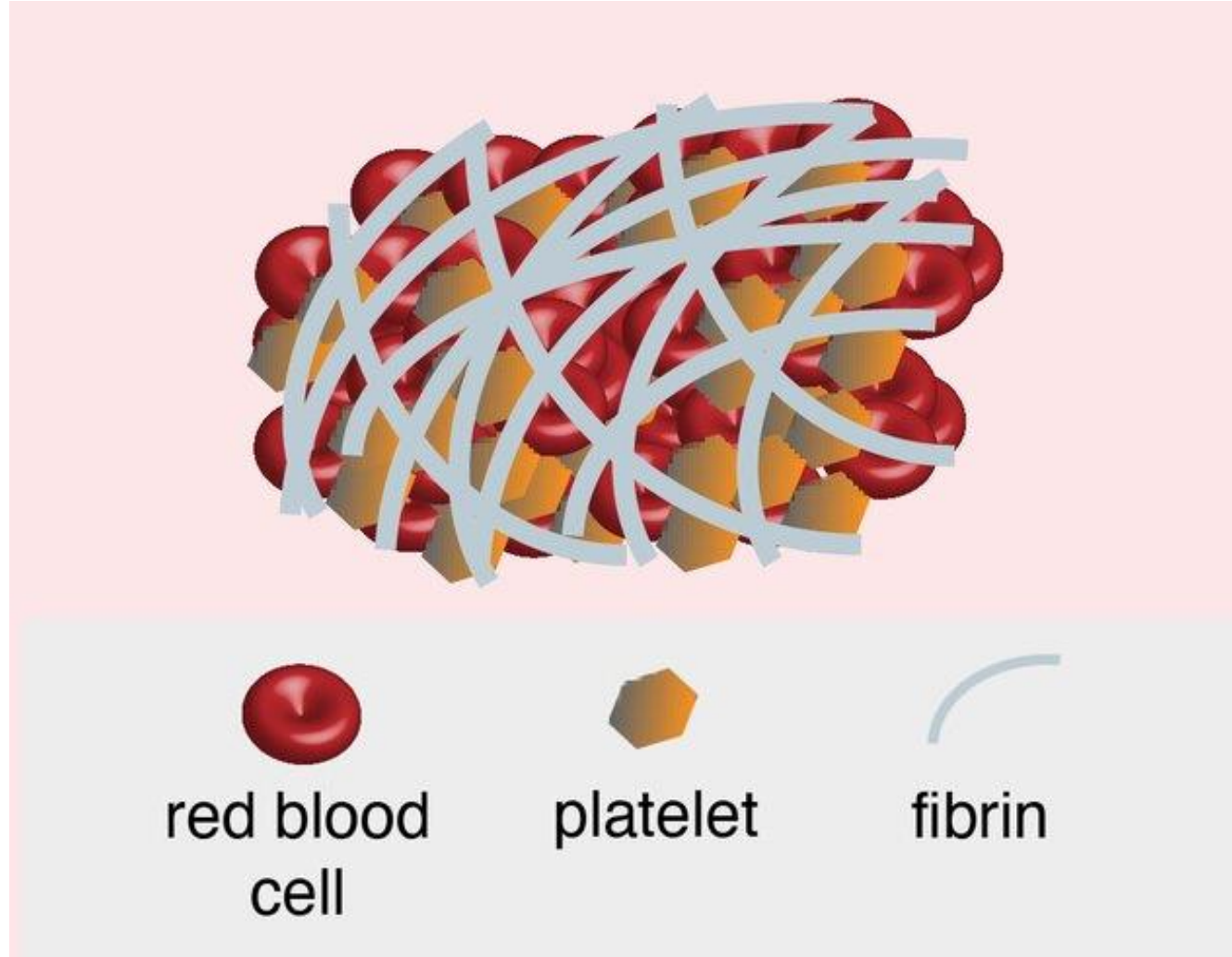


Steps of hemostasis and thrombosis

- Vascular constriction limiting blood flow to the area of injury
- Activation then aggregation of platelets at the site of injury, forming a loose platelet plug
- Formation of a fibrin mesh to entrap the plug
- Dissolution of the clot in order for normal blood flow to resume following tissue repair



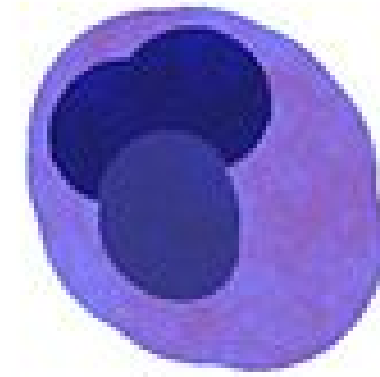
Composition of a clot



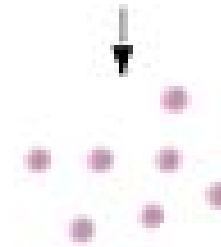


Platelets are a major player

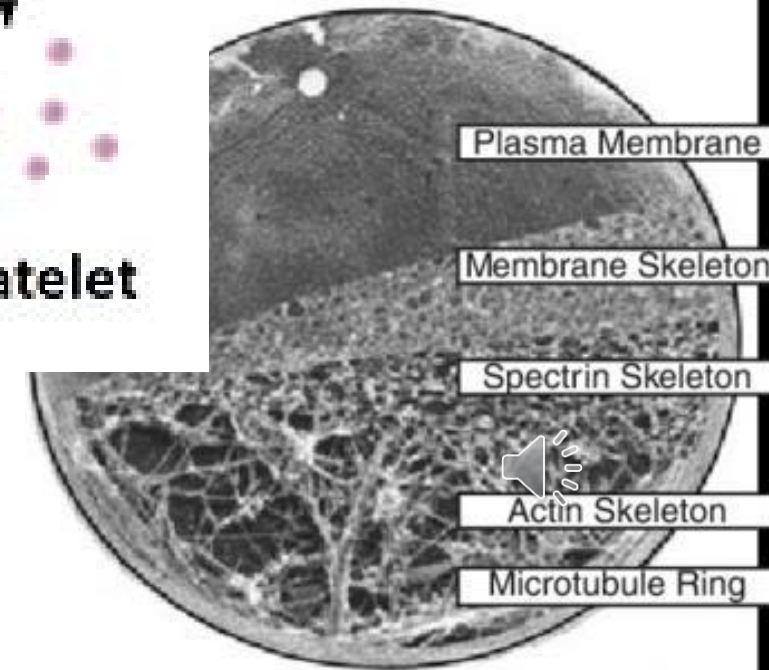
- Small anuclear cell fragments produced from the megakaryocytes.
- Platelets have numerous kinds of surface receptors.
- Platelets also have actin filaments and myosin, which change the shape of the platelet upon activation.
- They also have three types of granules that store substances that are released upon platelet activation.



Megakaryocyte

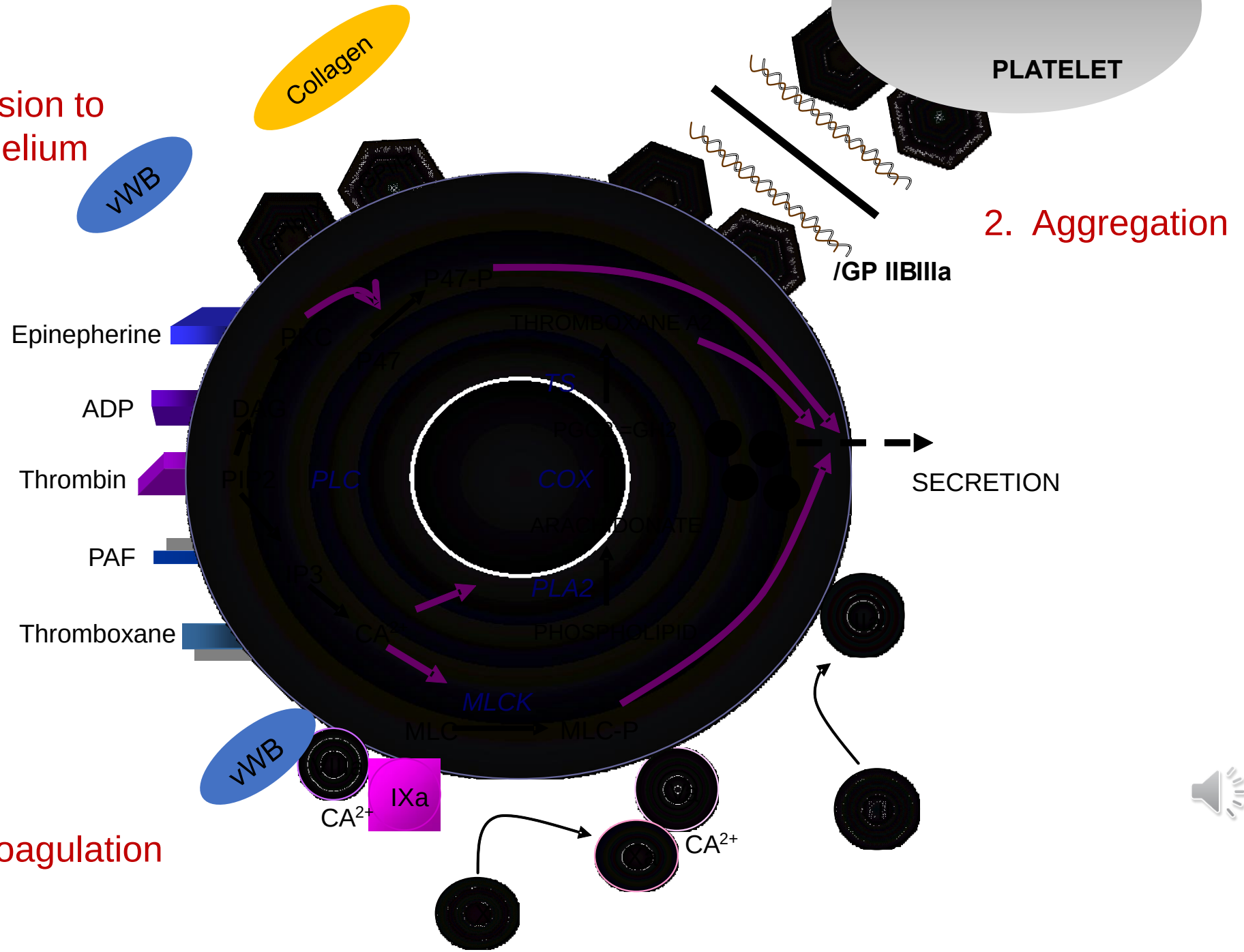


Platelet



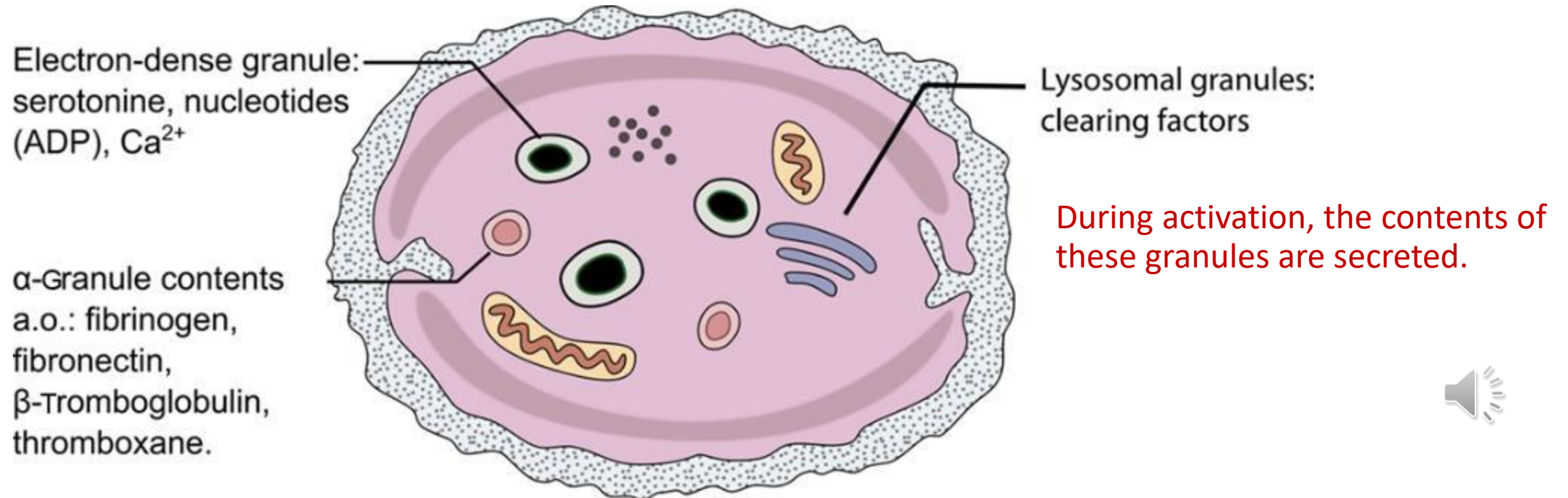


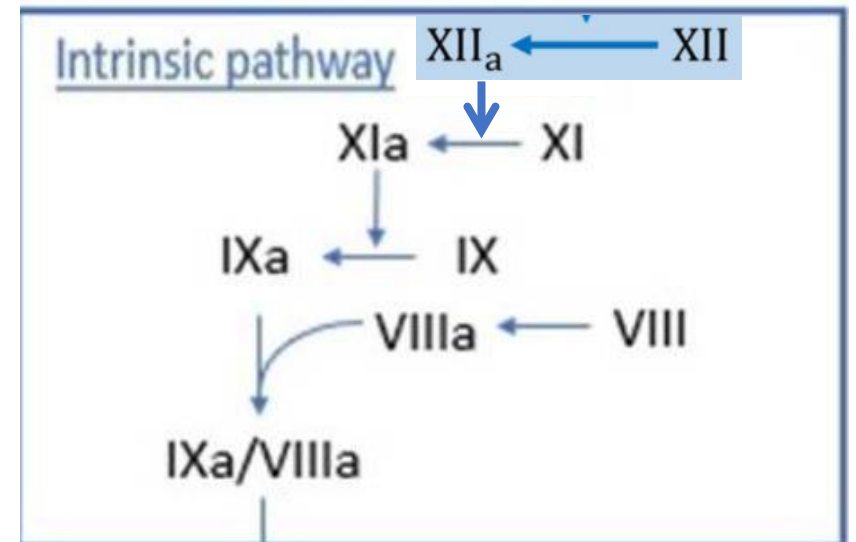
1. Adhesion to endothelium



The granules

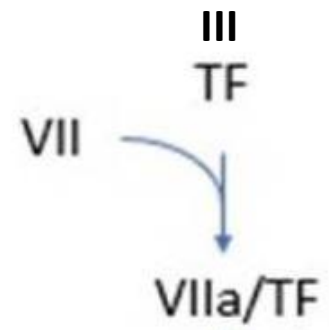
- Electron-dense granules (calcium ions, ADP, ATP, serotonin)
- α -granule (a heparin antagonist, platelet-derived growth factor, fibrinogen, von Willebrand factor (vWF), clotting factors)
- Lysosomal granules (hydrolytic enzymes)



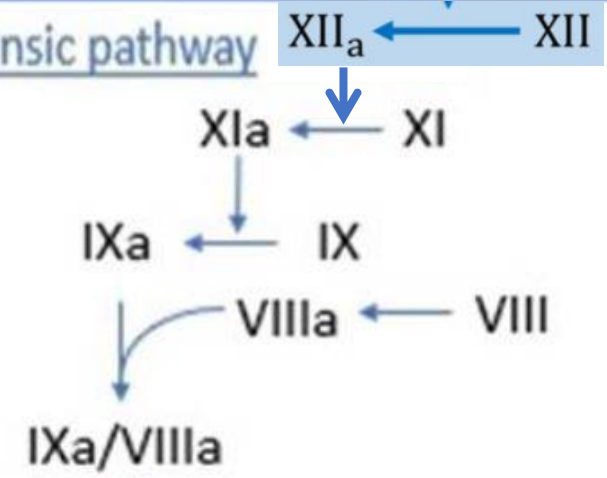


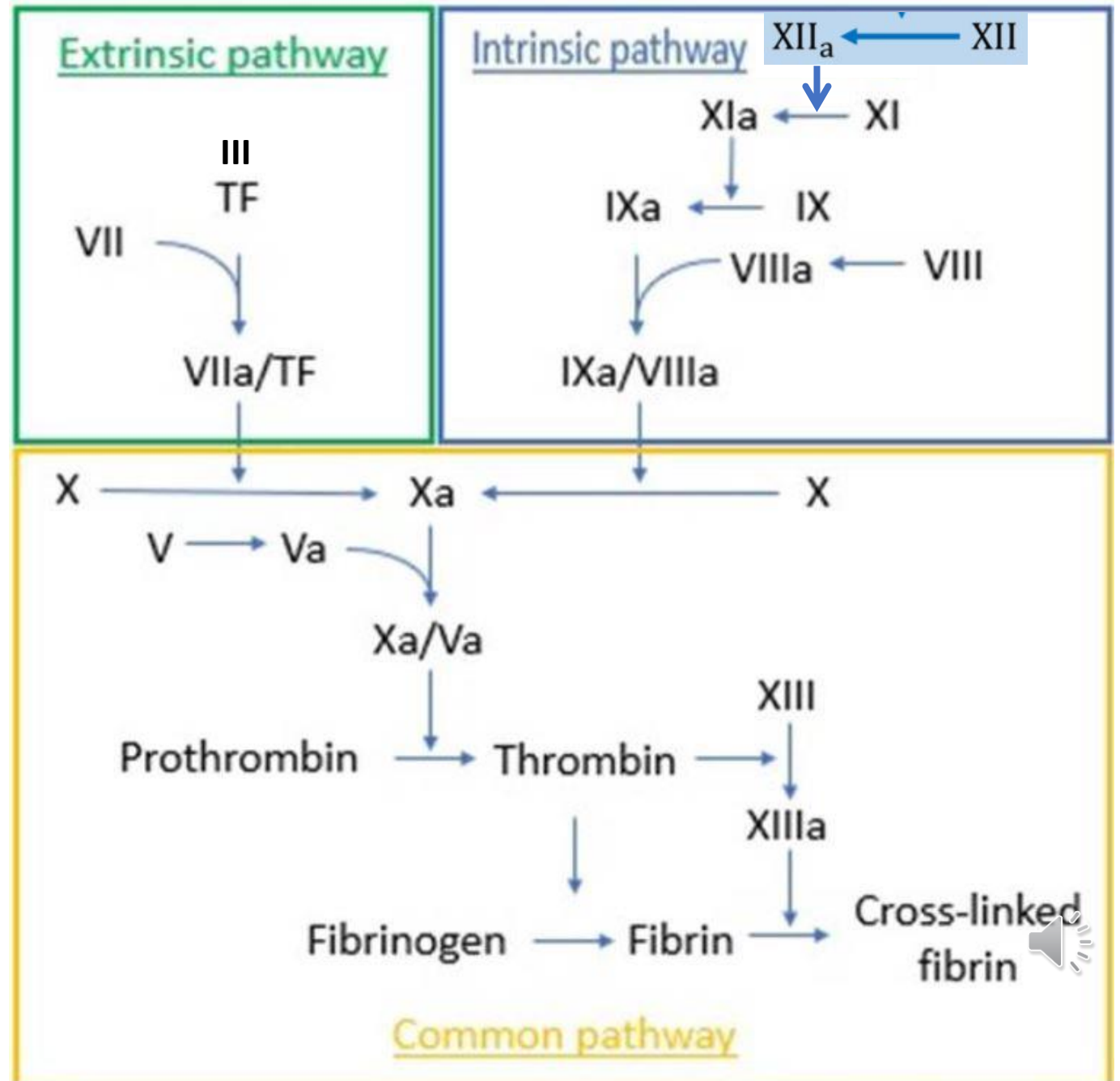


Extrinsic pathway



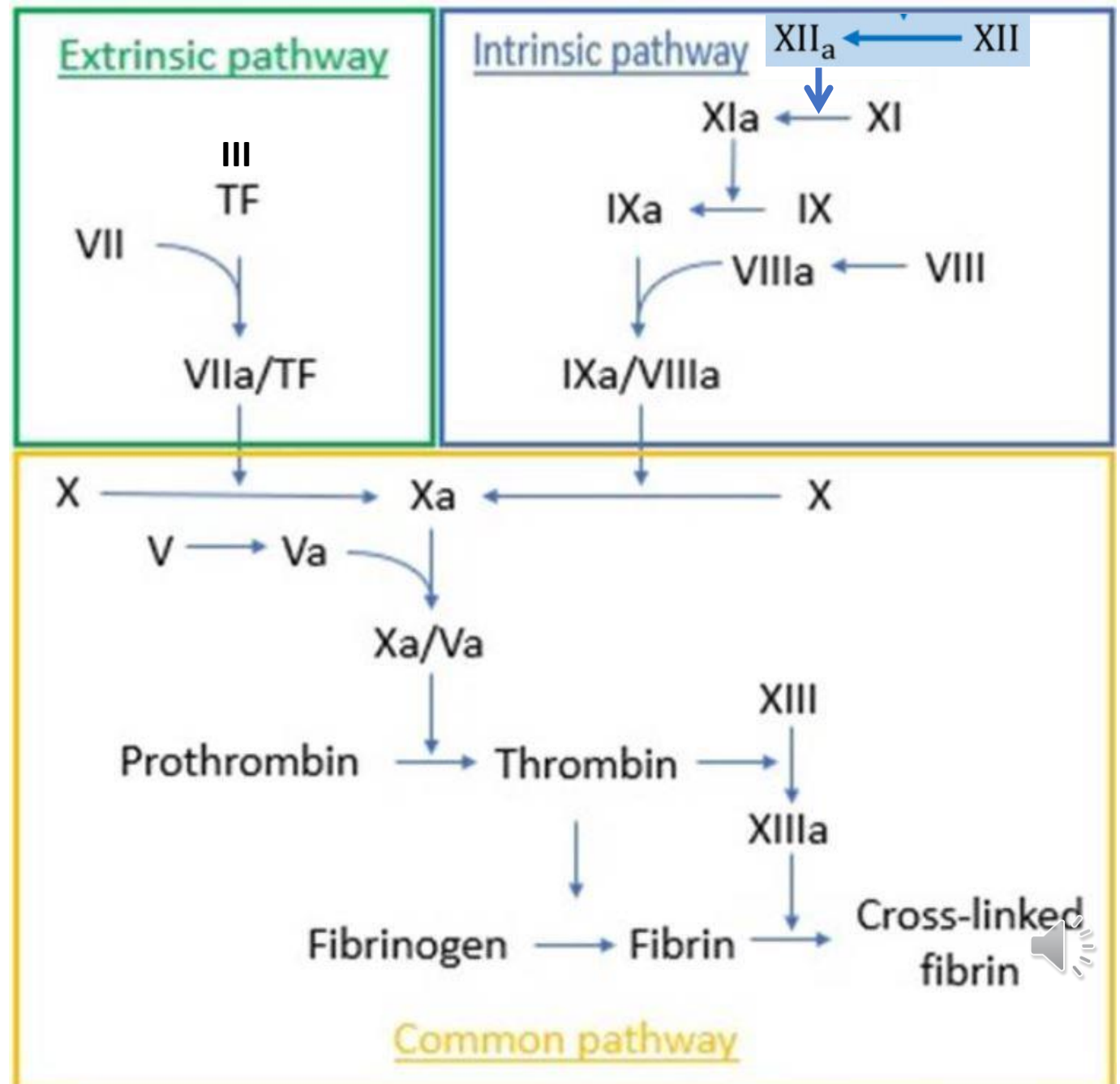
Intrinsic pathway

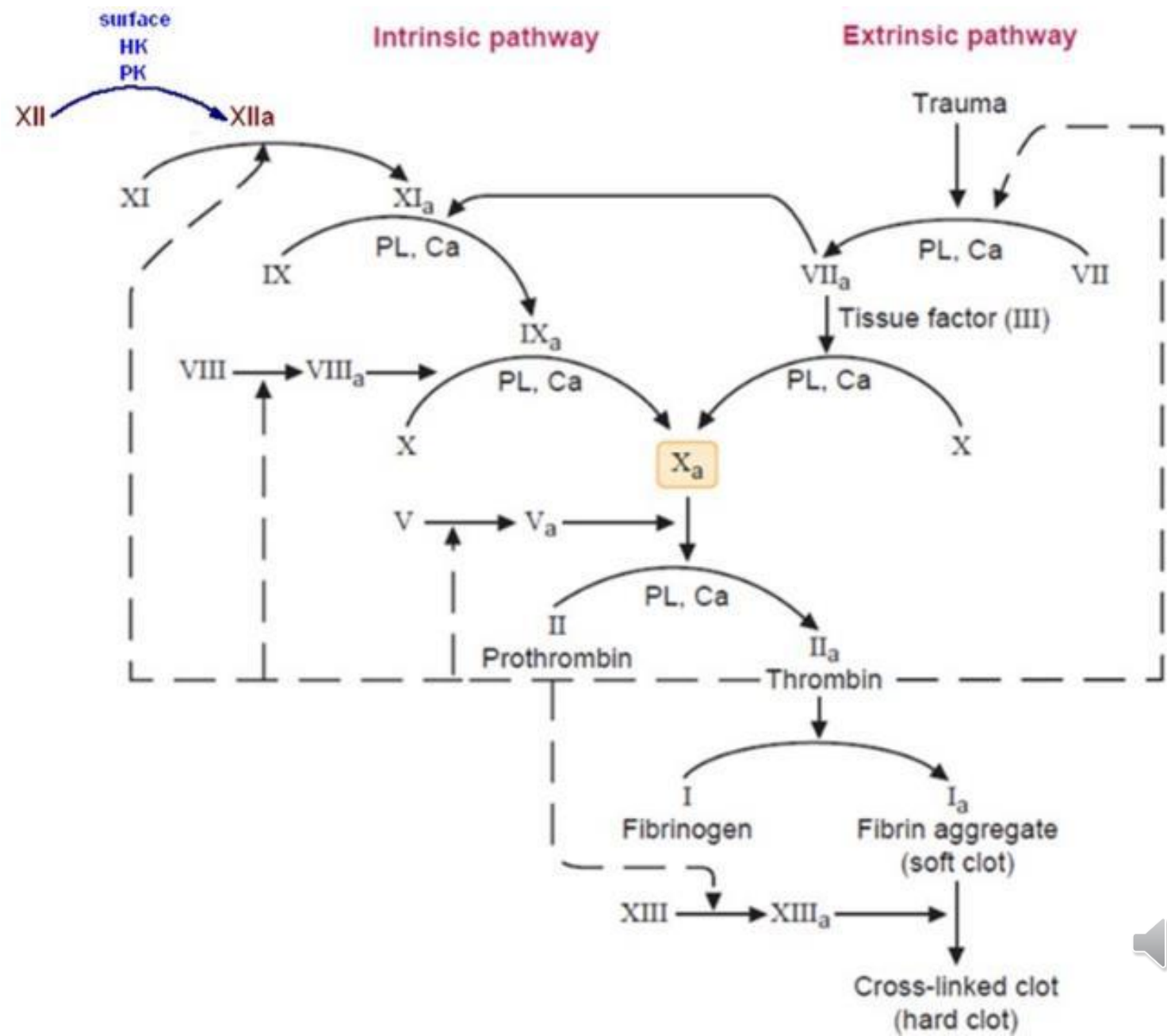
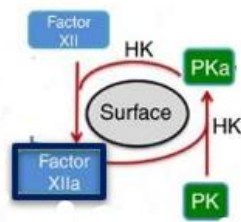


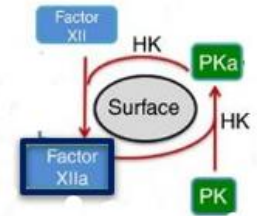


-Important notes:

- 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.

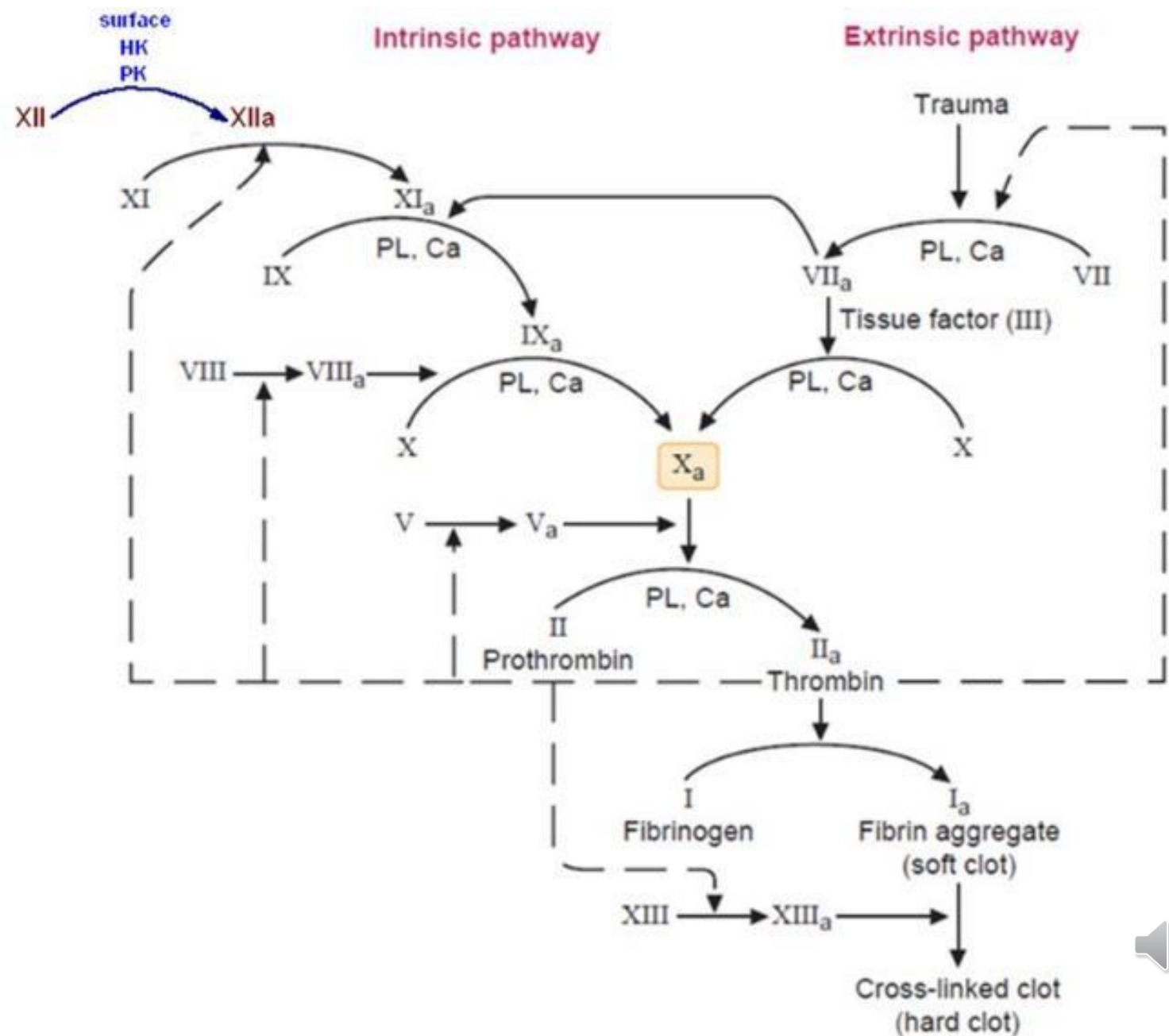


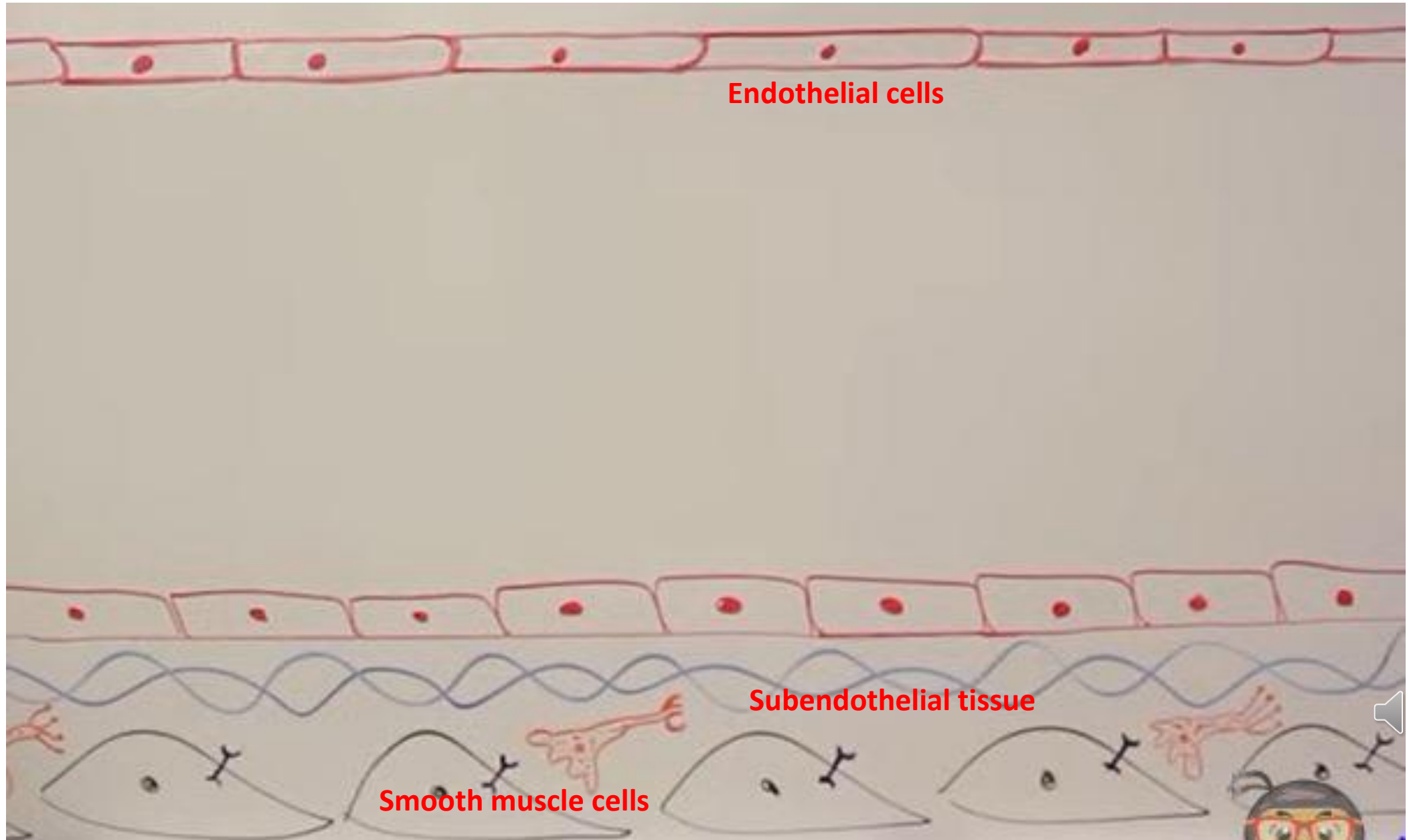


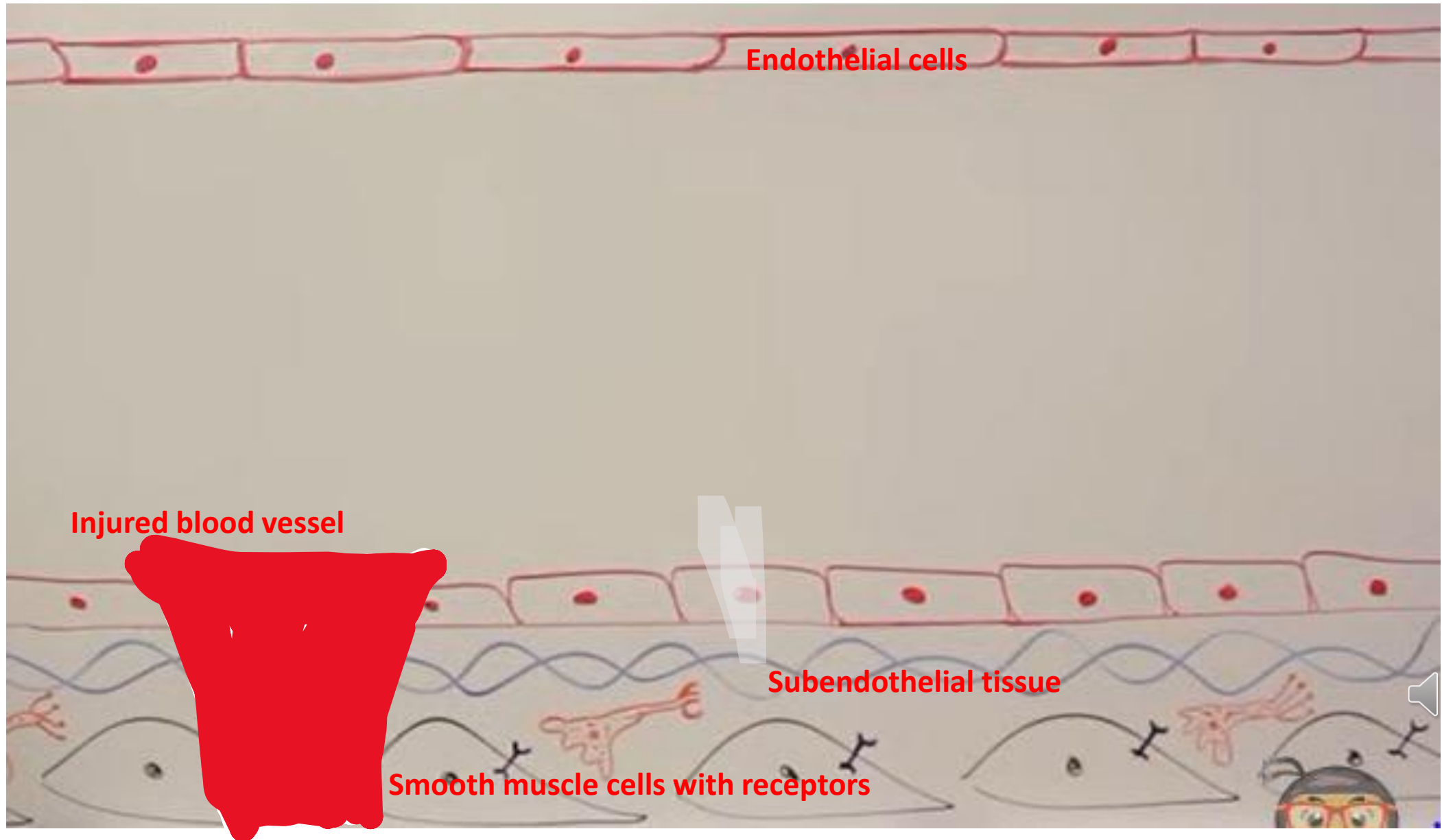


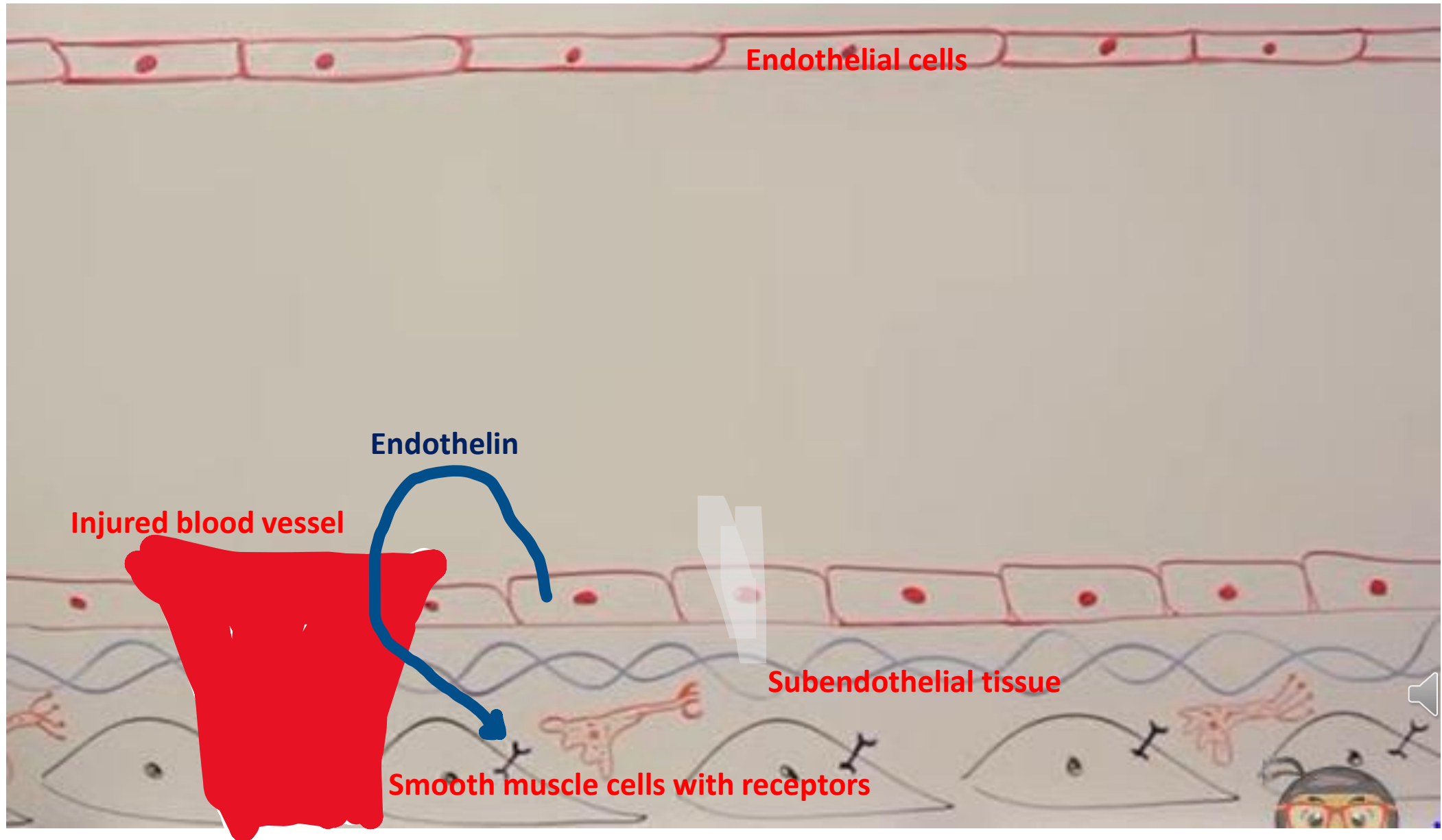
-Important notes:

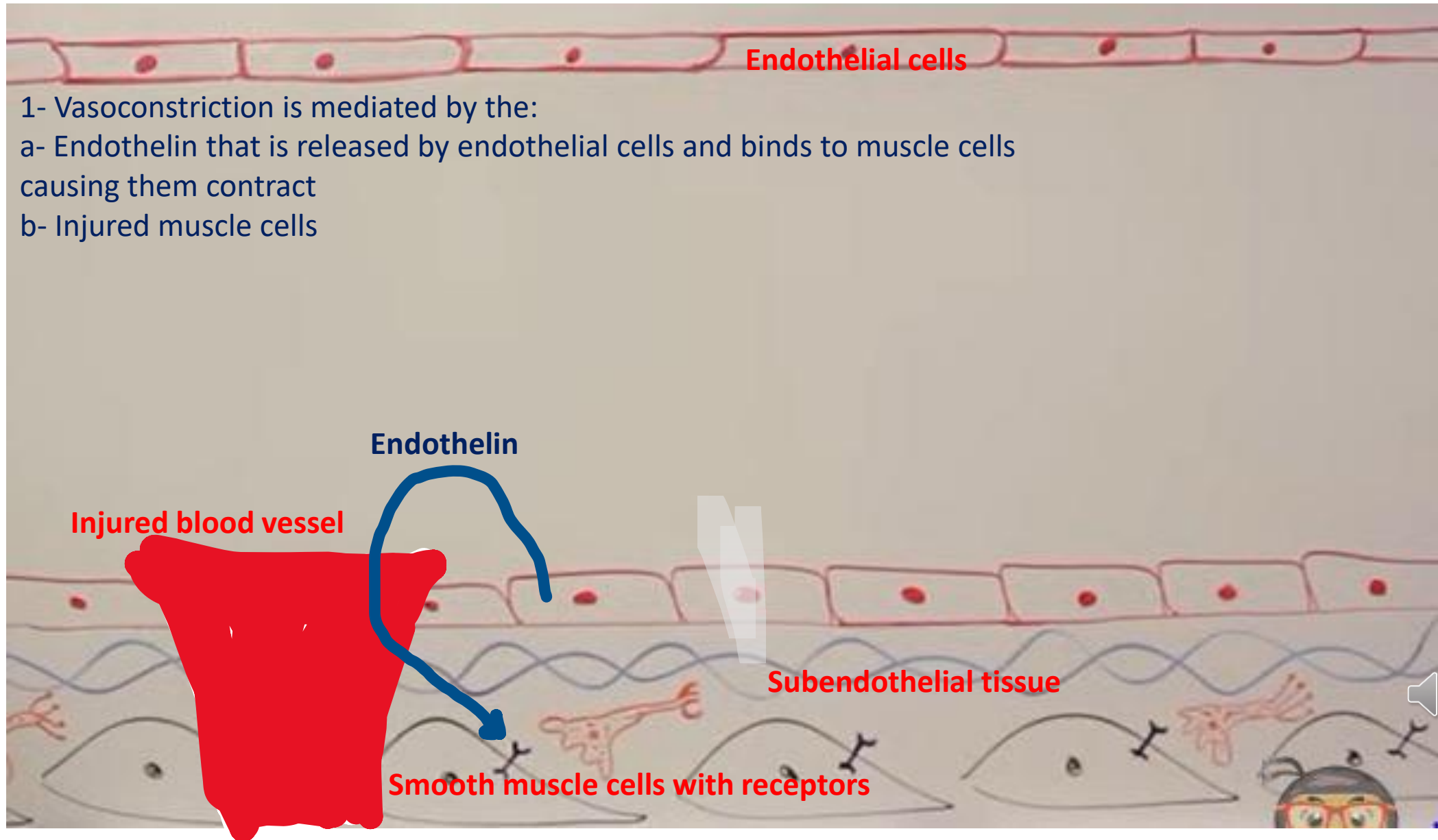
- 1- Factors 2,7,9 and 10 (the major players) require Ca^{+2} , vitamin K and platelets surface.
- 2- Notice the connection between factor 7 and 9, connecting the extrinsic pathway to the intrinsic pathway.
- 3- Thrombin (factor 2): Does a positive feedback loop to factors 7,11, 5 and 8.
- 4- Factor 12 performs a positive feedback loop with Prekallekrein and activates it to Kallekrein, which itself reactivates factor 12.







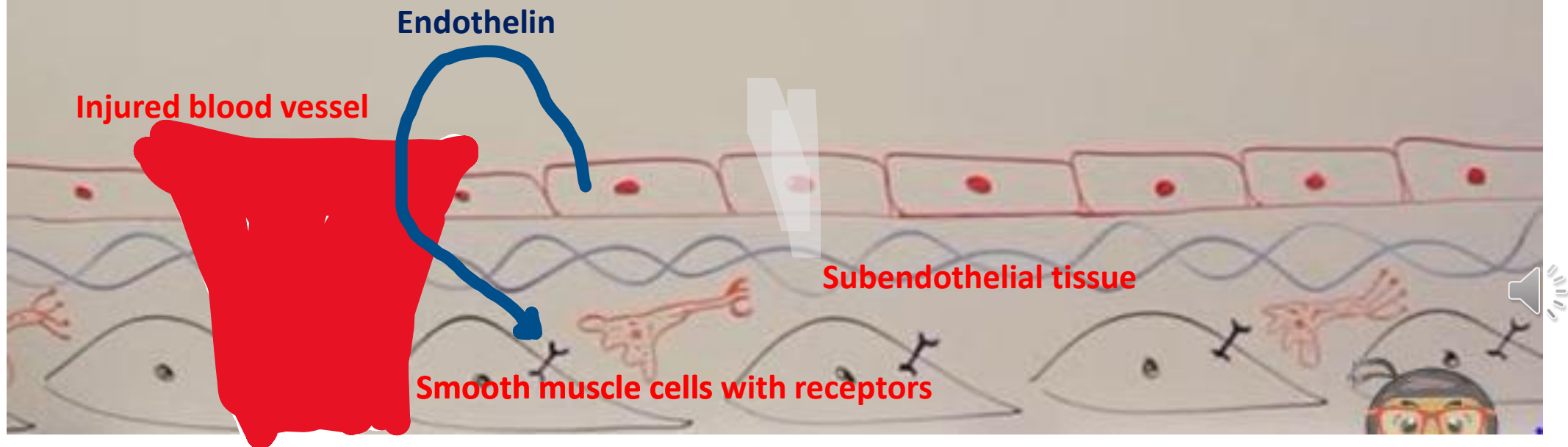






Endothelial cells

- 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.





Endothelial cells

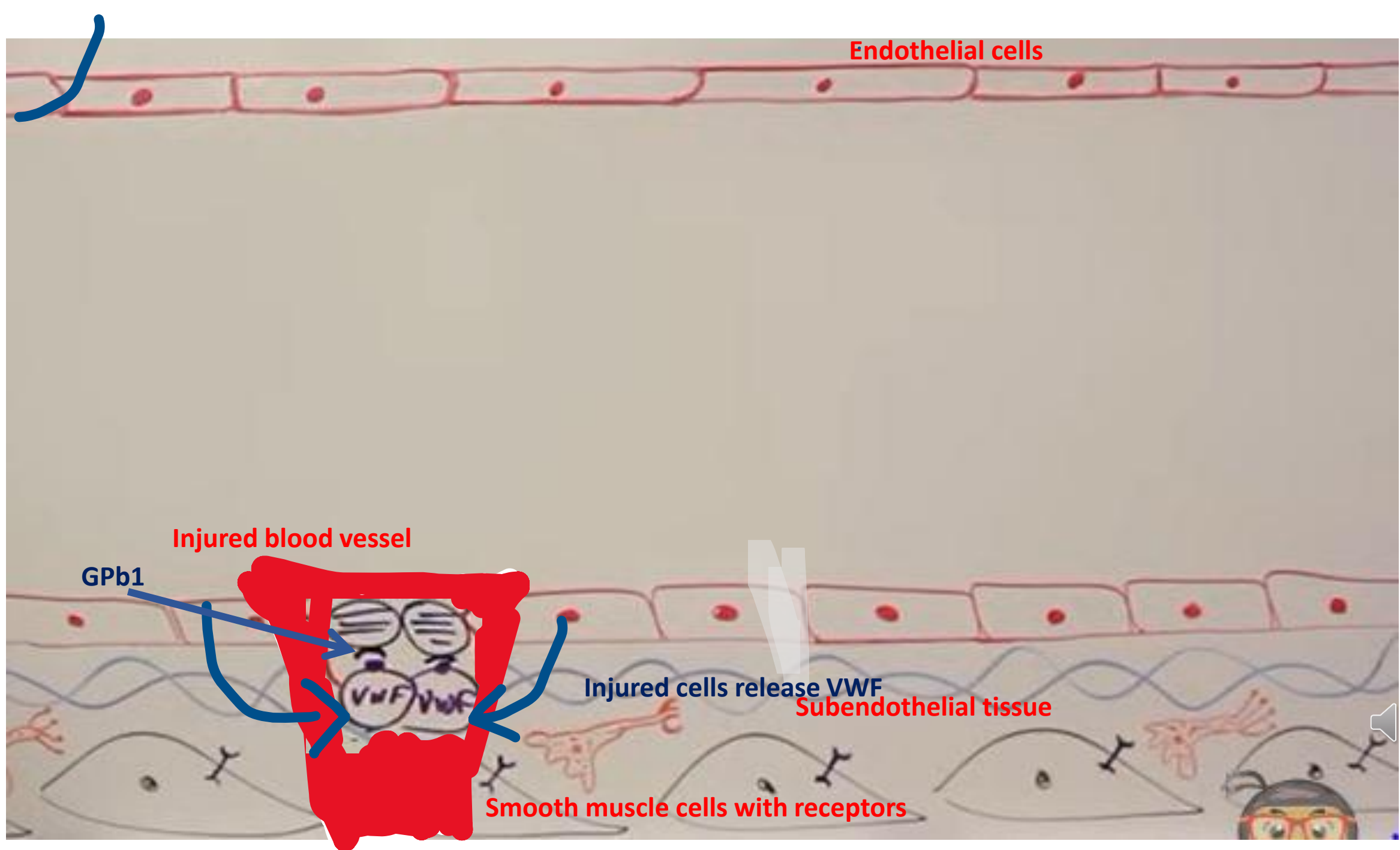
Injured blood vessel

GPb1

Injured cells release VWF

Subendothelial tissue

Smooth muscle cells with receptors





Endothelial cells

Injured blood vessel

GPb1

Fibrinogen

Serotonin

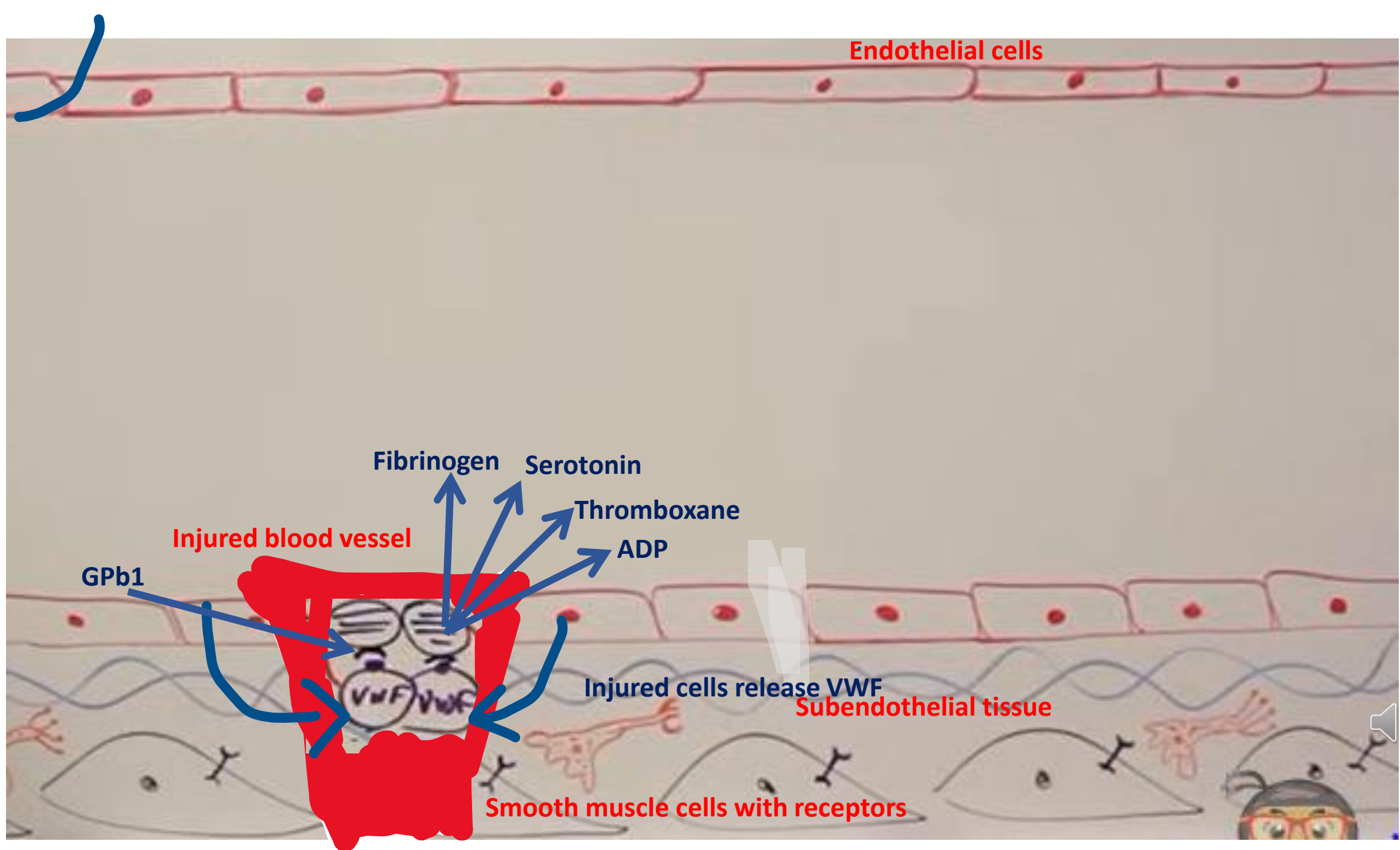
Thromboxane

ADP

Injured cells release VWF

Subendothelial tissue

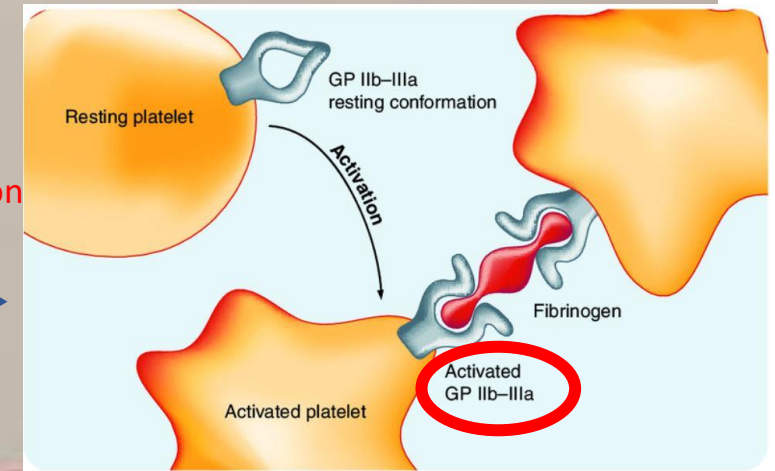
Smooth muscle cells with receptors





Endothelial cells

The signals lead to platelet aggregation and change in their shape



Injured blood vessel

GPb1

Fibrinogen

Serotonin

Thromboxane

ADP

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.

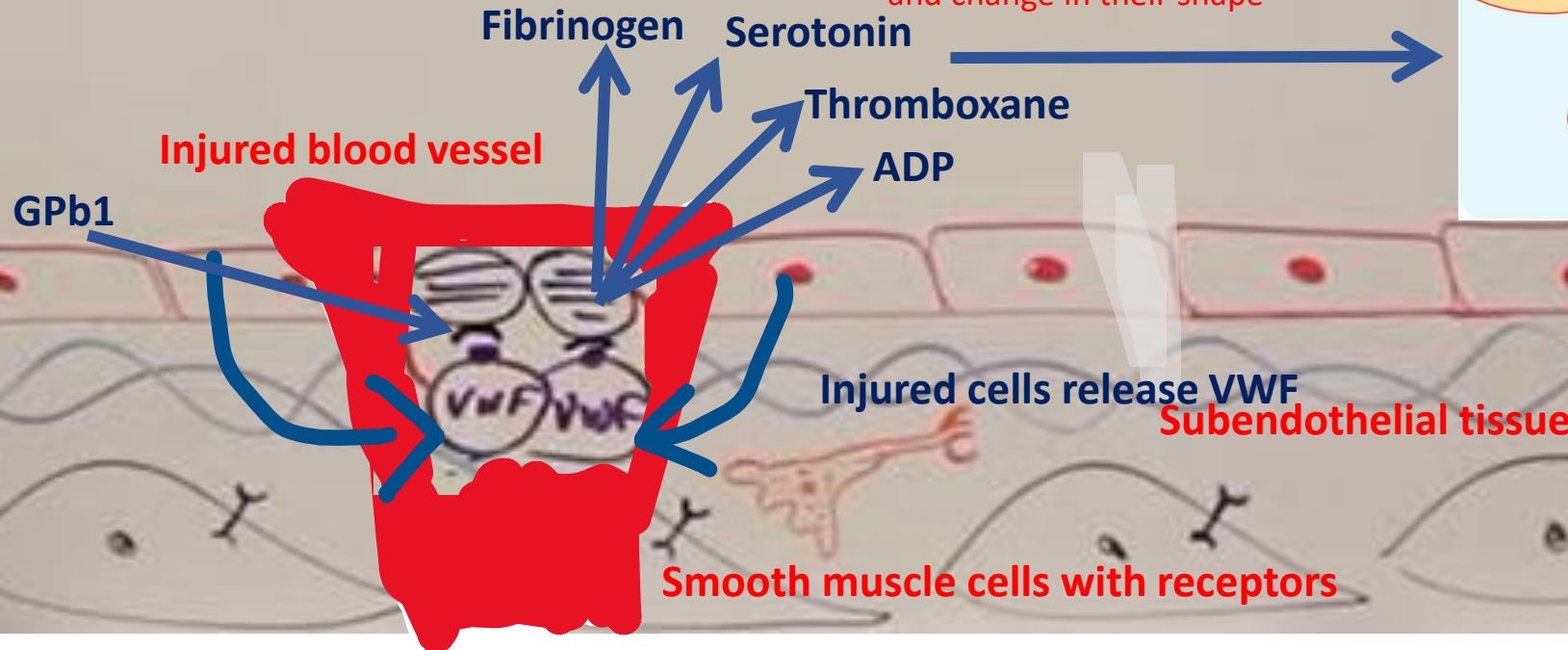
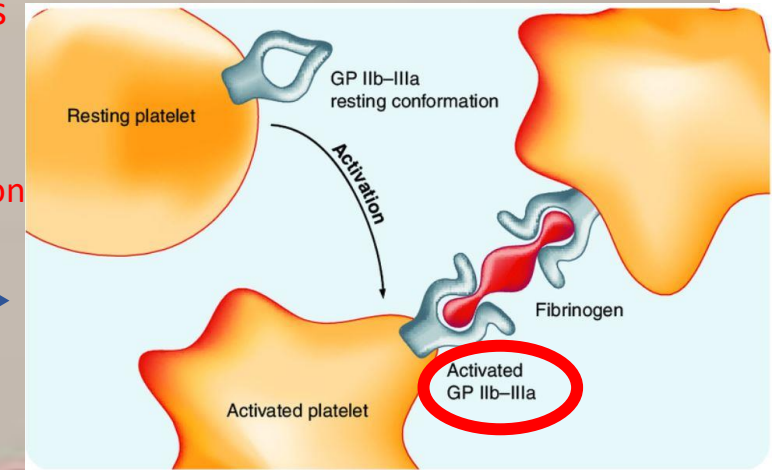
1- ADP is the **key signal in aggregating the platelets** and is recognized by G proteins P2Y1 and P2Y12 receptors on the other platelets. This signal upregulates proteins GP2b and GP3a on the surface of the platelets allowing them to bind to each others (aggregate). The signal also induces the platelets 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.

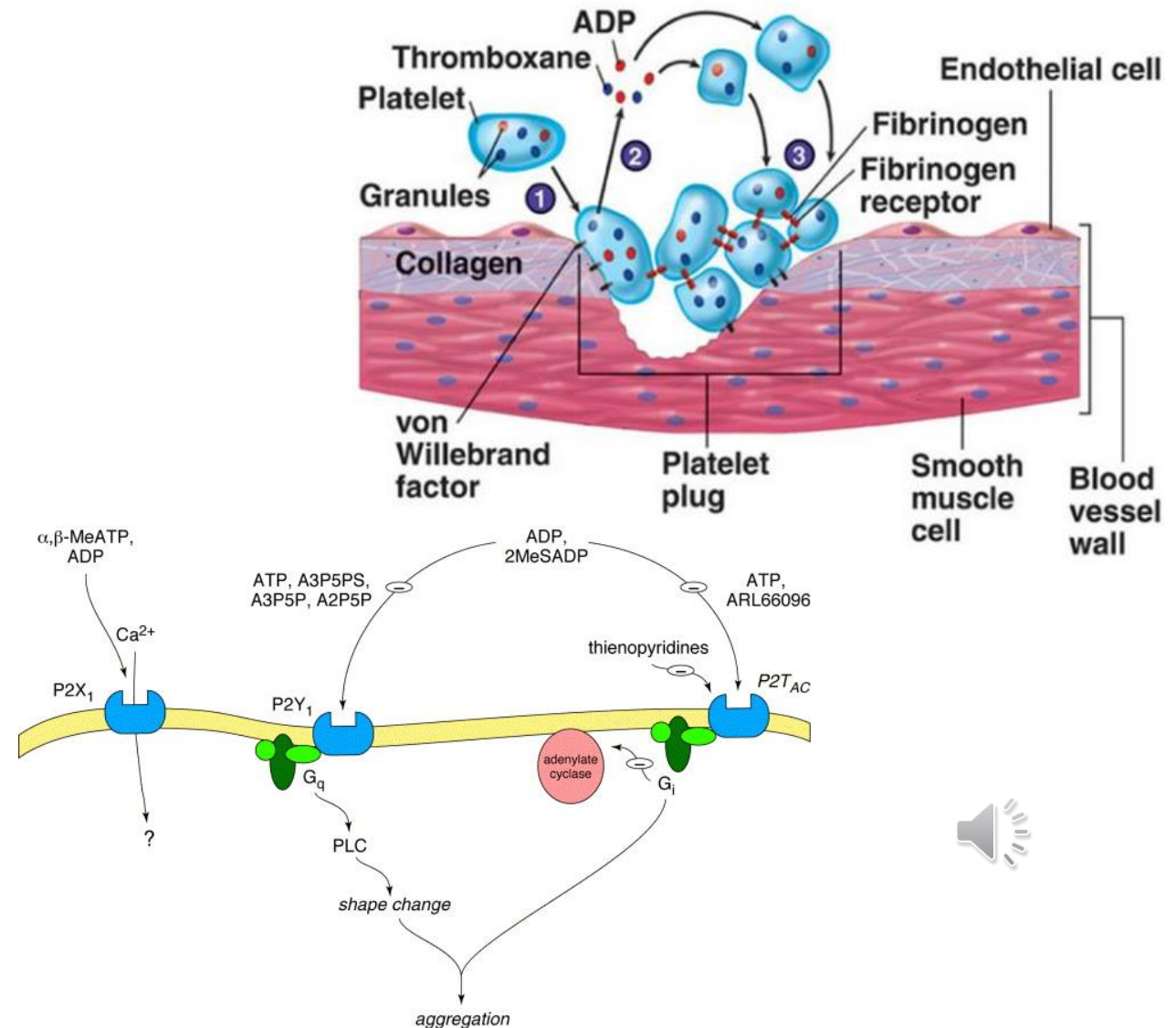
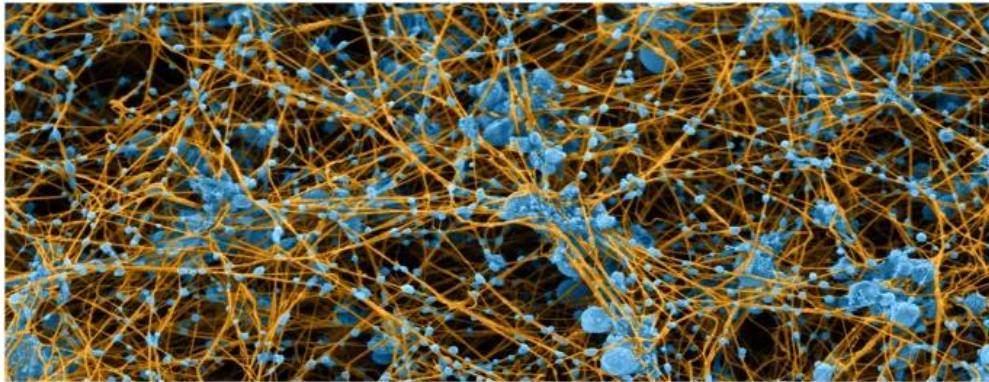
The signals lead to platelet aggregation and change in their shape





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.

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

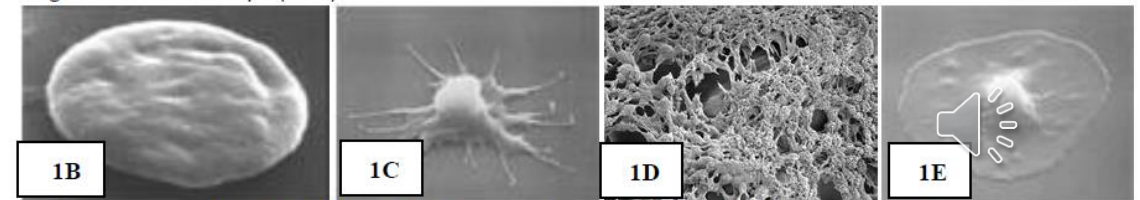
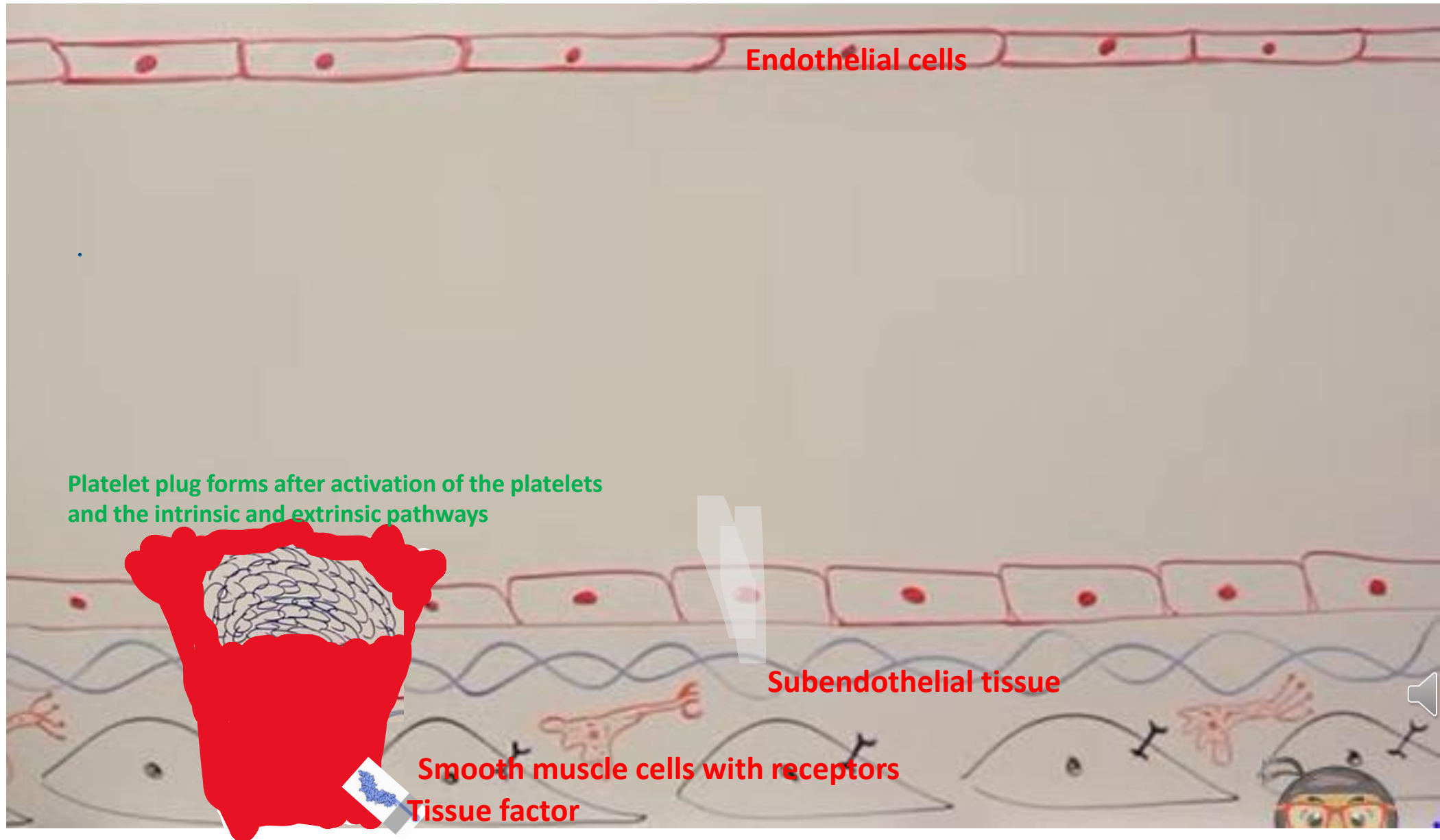


Figure 1 [(B) Platelet in resting mode (C) Activated platelets change into a pseudopodia shape (D) Aggregated platelets (E) Platelet spreading]



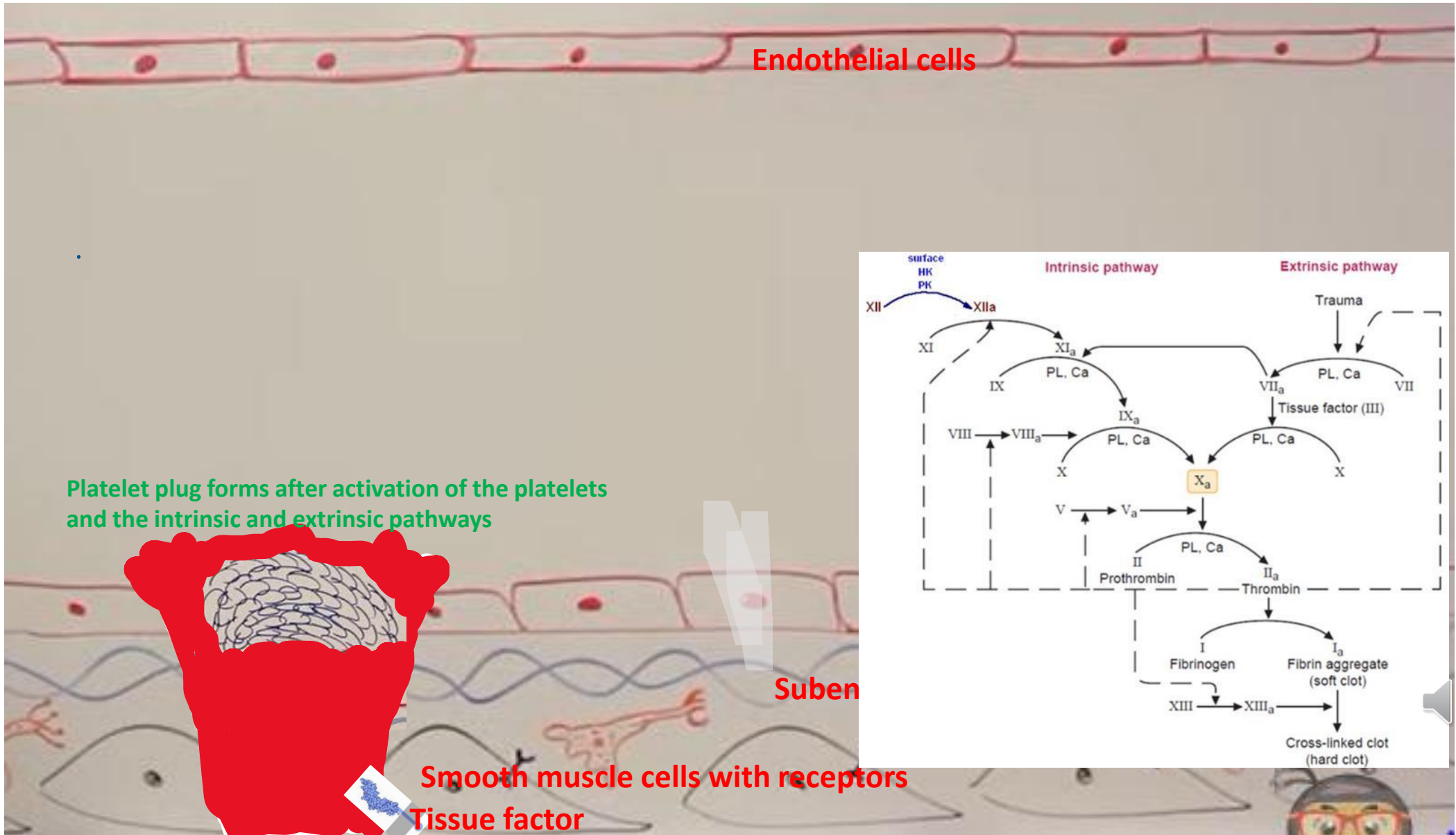
Endothelial cells

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

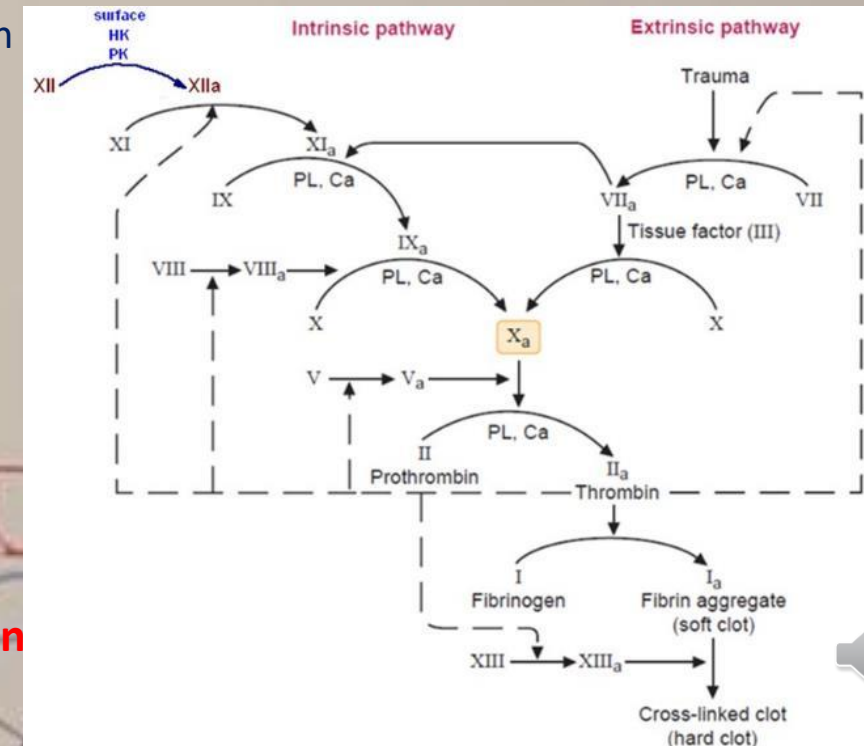
- In parallel to platelet activation, the extrinsic and the intrinsic pathways are also activated. The extrinsic pathway is activated only if the tissue factor is exposed. The extrinsic pathway is activated in approximately 10 seconds. The intrinsic pathway is activated in 4-6 minutes.
- If the extrinsic 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



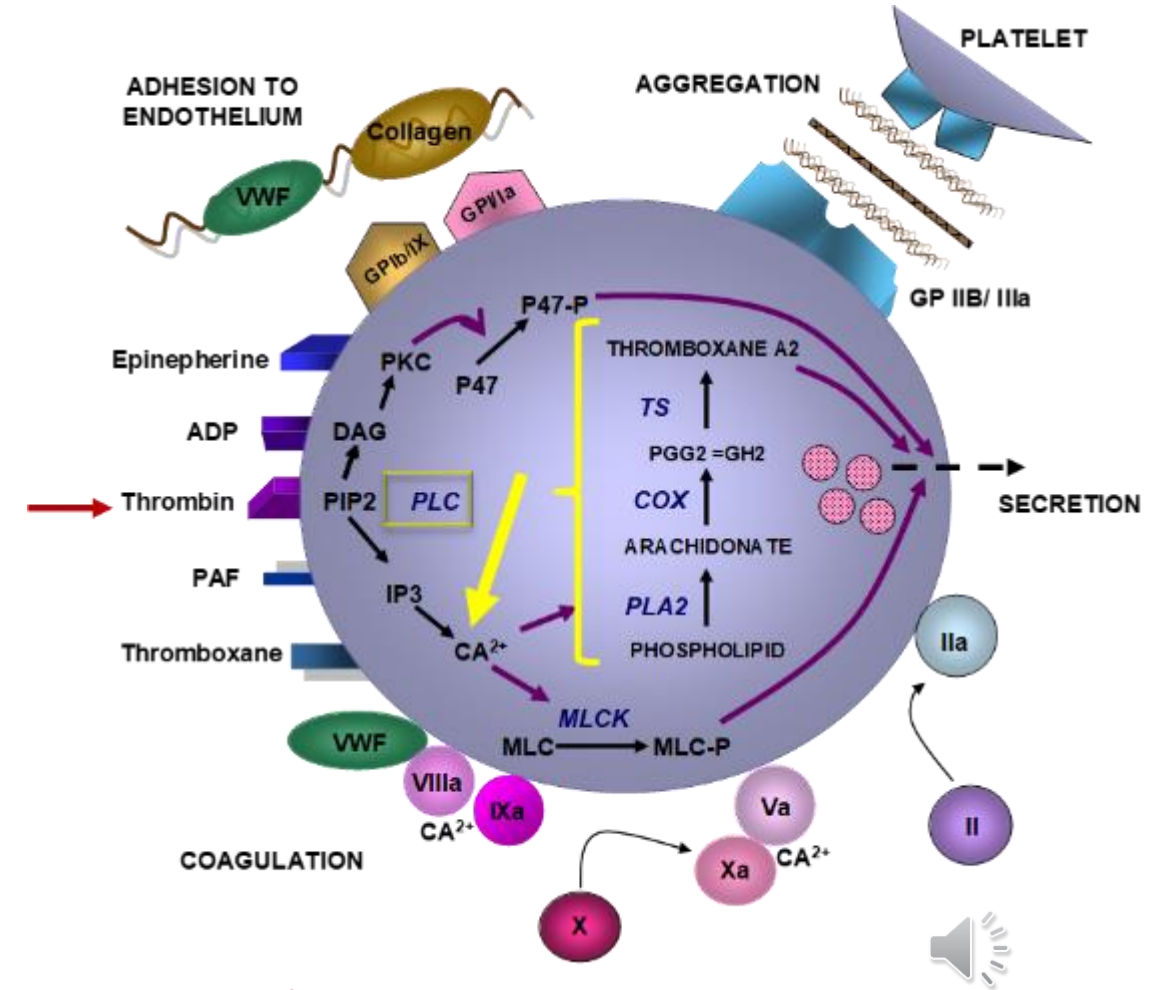


- **We will dive a little deeper into the role of thrombin in signaling to platelets. We will also discuss the coagulation cascade in more details.**



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 Ca²⁺ stores, and DAG activates protein kinase C (PKC).
- Ca²⁺ 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.

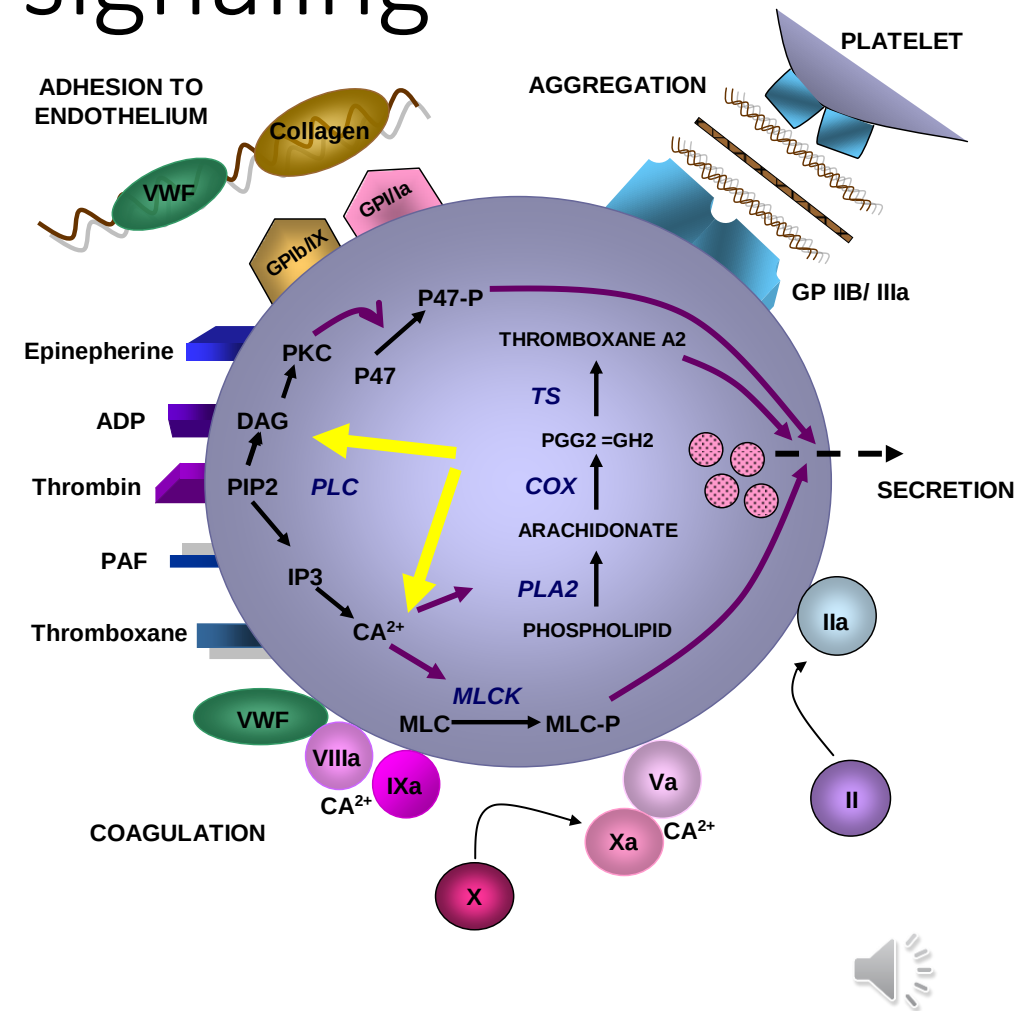


- *Serotonin is also a vasoconstrictor.*
- *PDGF stimulates proliferation of endothelial cells to reduce blood flow.*



More release of Thrombin signaling

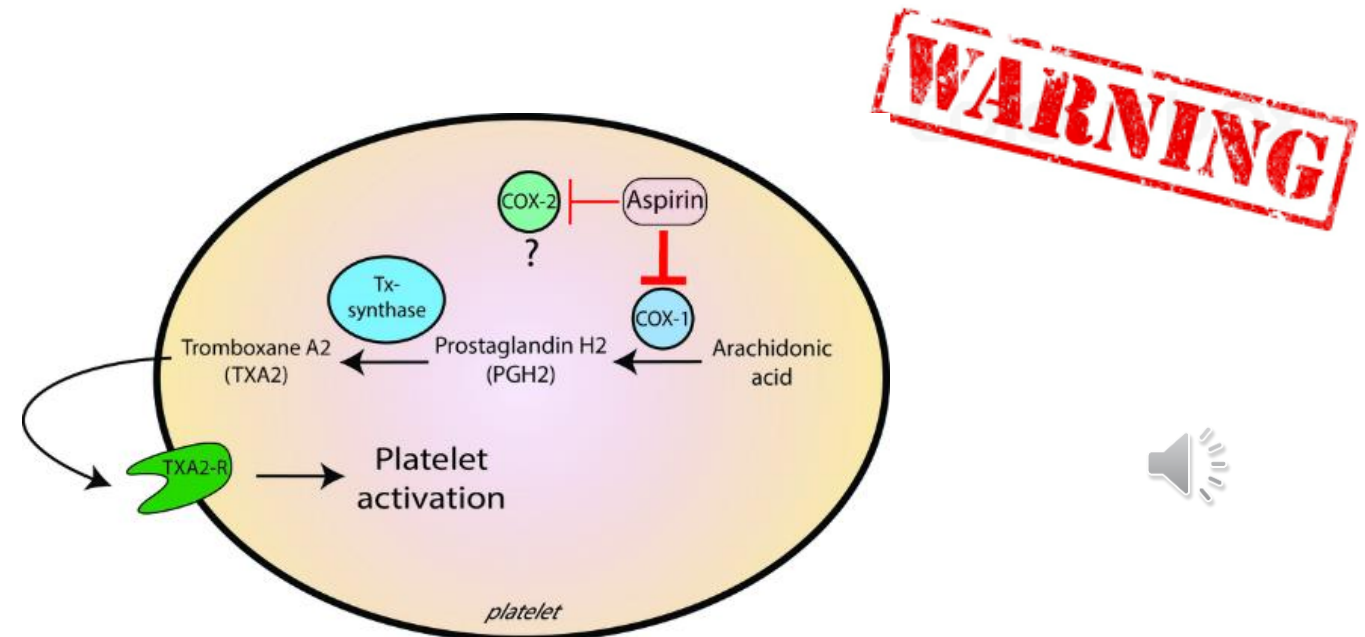
- Ca^{2+} activates myosin light chain kinase (MLCK), which phosphorylates the light chain of myosin allowing it to interact with actin and resulting in altered platelet morphology, induced motility, and release of granules.
- DAG activates PKC, which phosphorylates and activates specific platelet proteins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



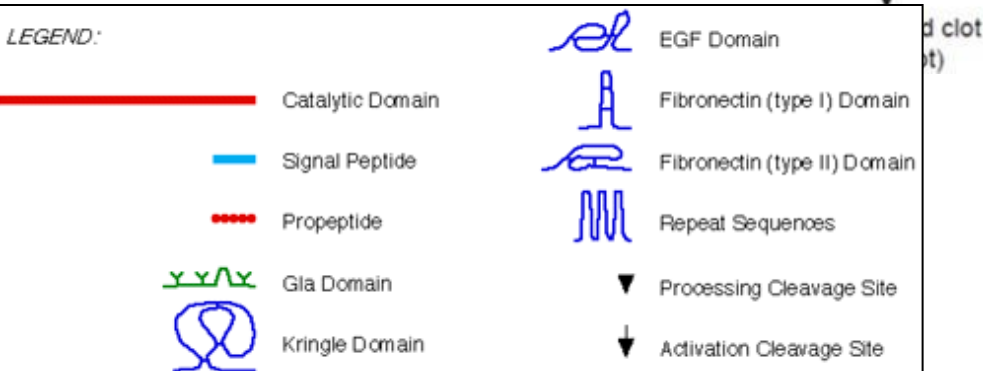
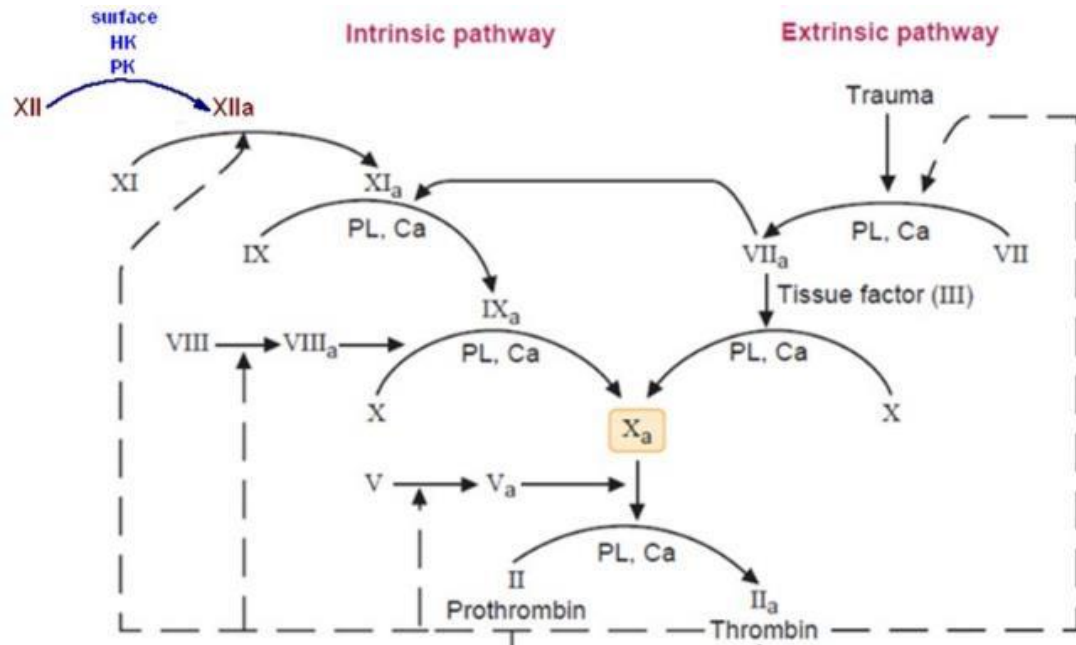


NSAID

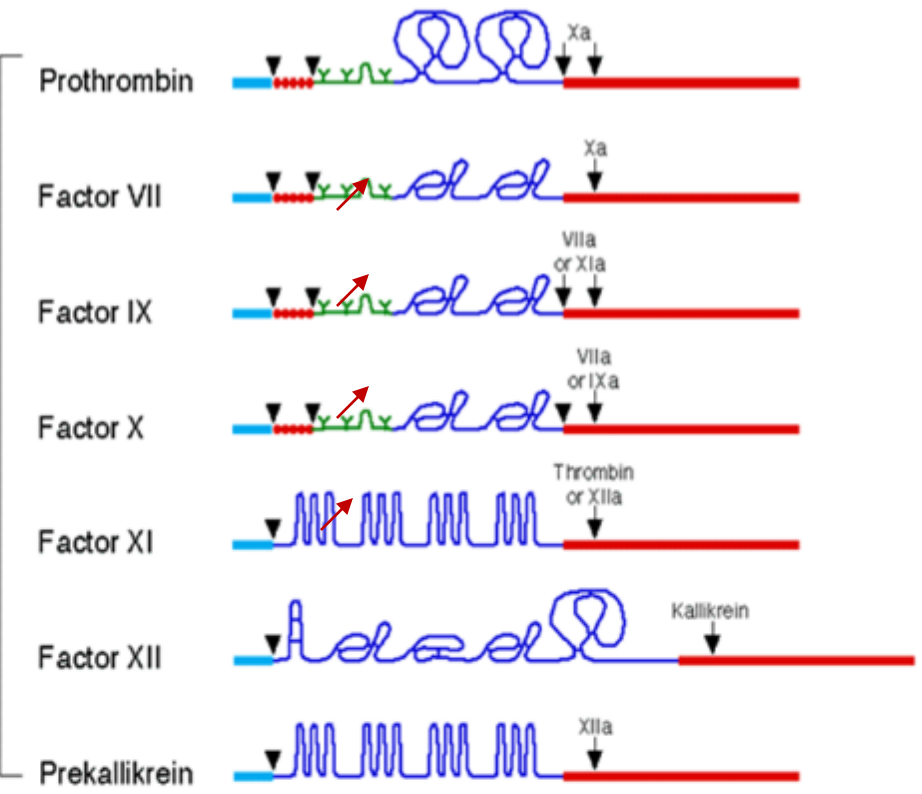
- Non-steroidal anti-inflammatory drugs inhibit the cyclooxygenase (inhibiting prostaglandin and thromboxane) accounting for their anticoagulant effects.
 - Aspirin also inhibits production of endothelial prostacyclin, which opposes platelet aggregation and is a vasodilator, but, unlike platelets, these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



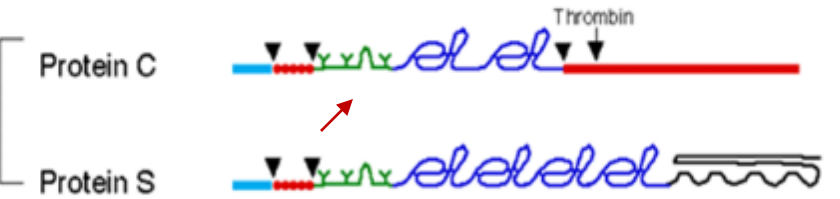
Coagulation cascade



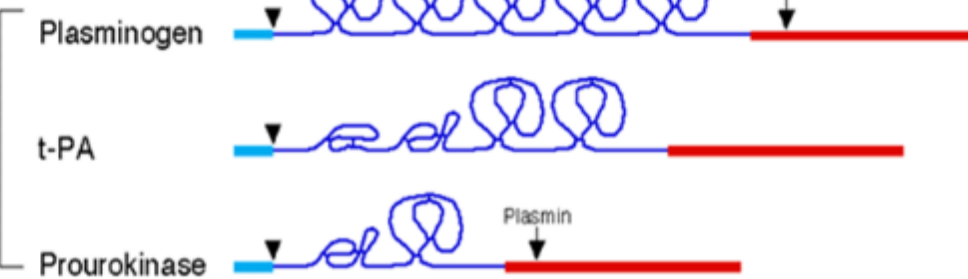
Procoagulant Factors



Anticoagulant Factors

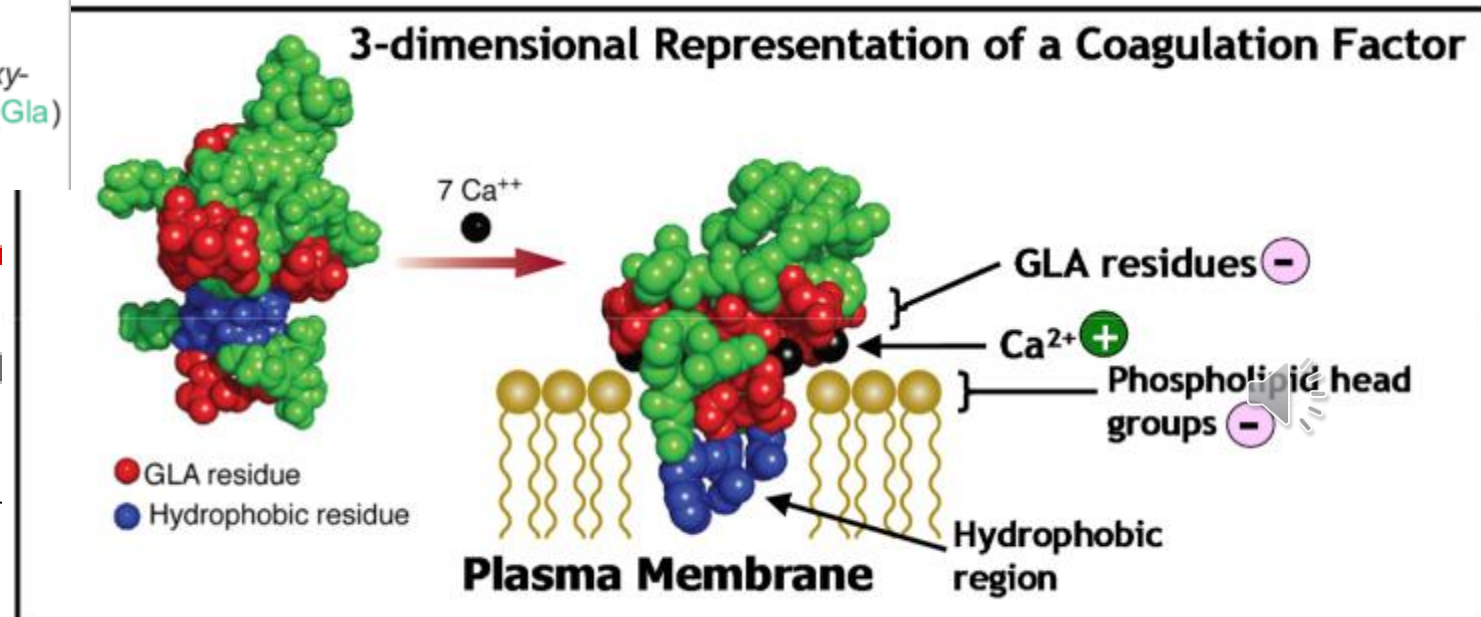
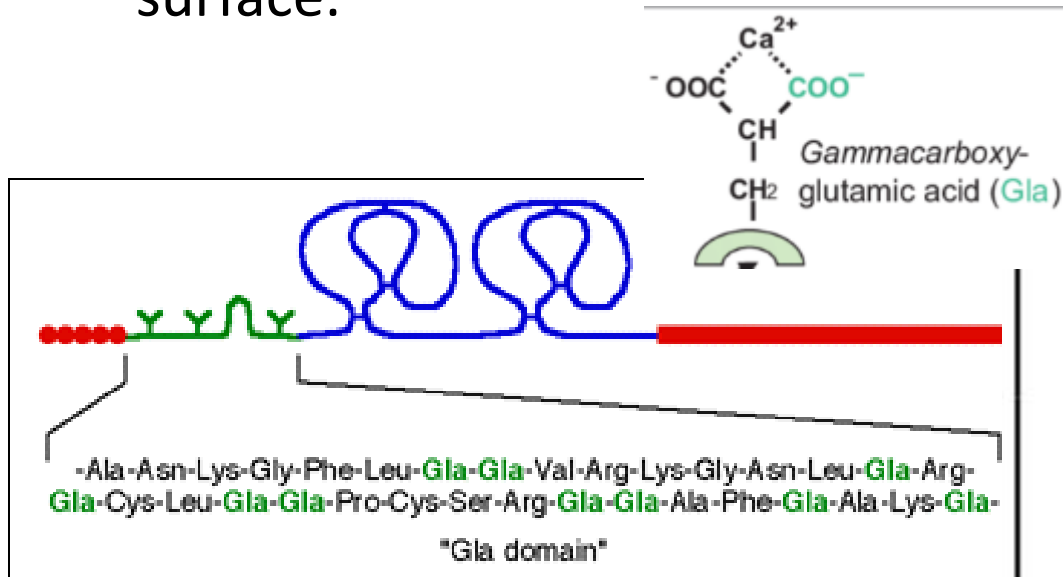


Fibrinolytic Factors



Gla domain

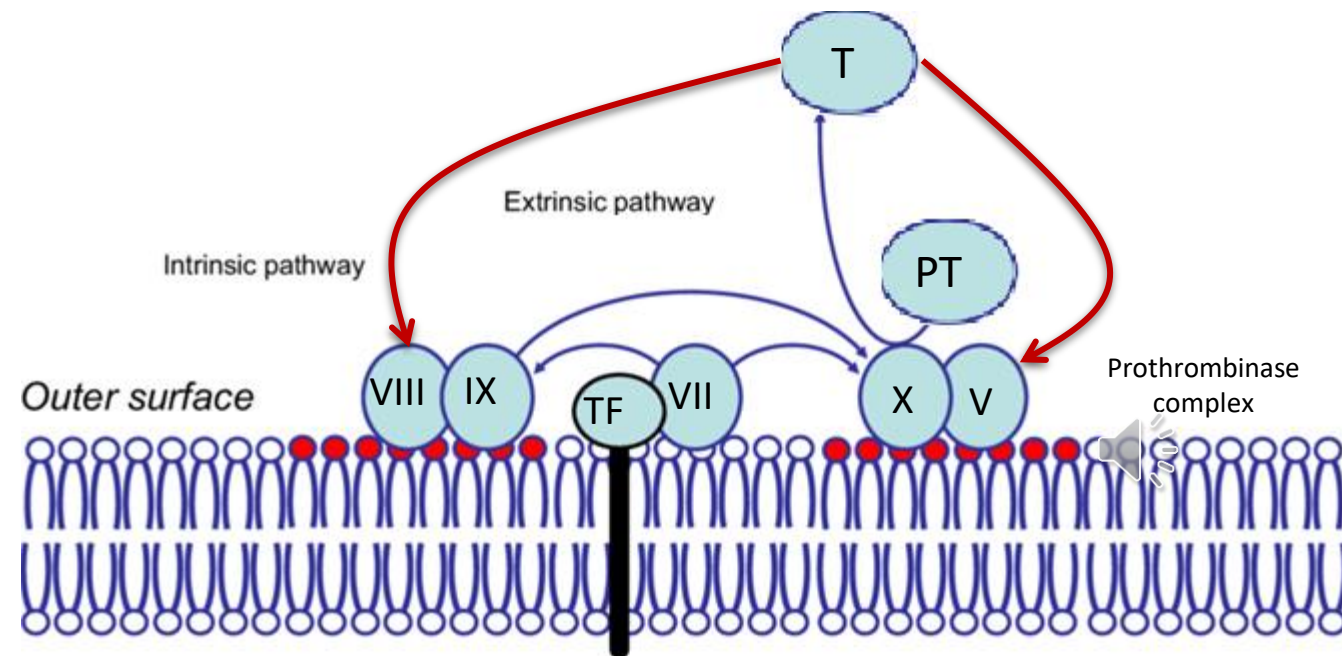
- An ER/Golgi carboxylase binds to prothrombin and factors IX, VII, and X and converts 10≥ glutamate (Glu) residues to γ -carboxyglutamate (Gla), followed by a small (10 a.a.) hydrophobic region.
- The Gla residues bind calcium ions and are necessary for the activity of these coagulation factors and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





The tenase complexes

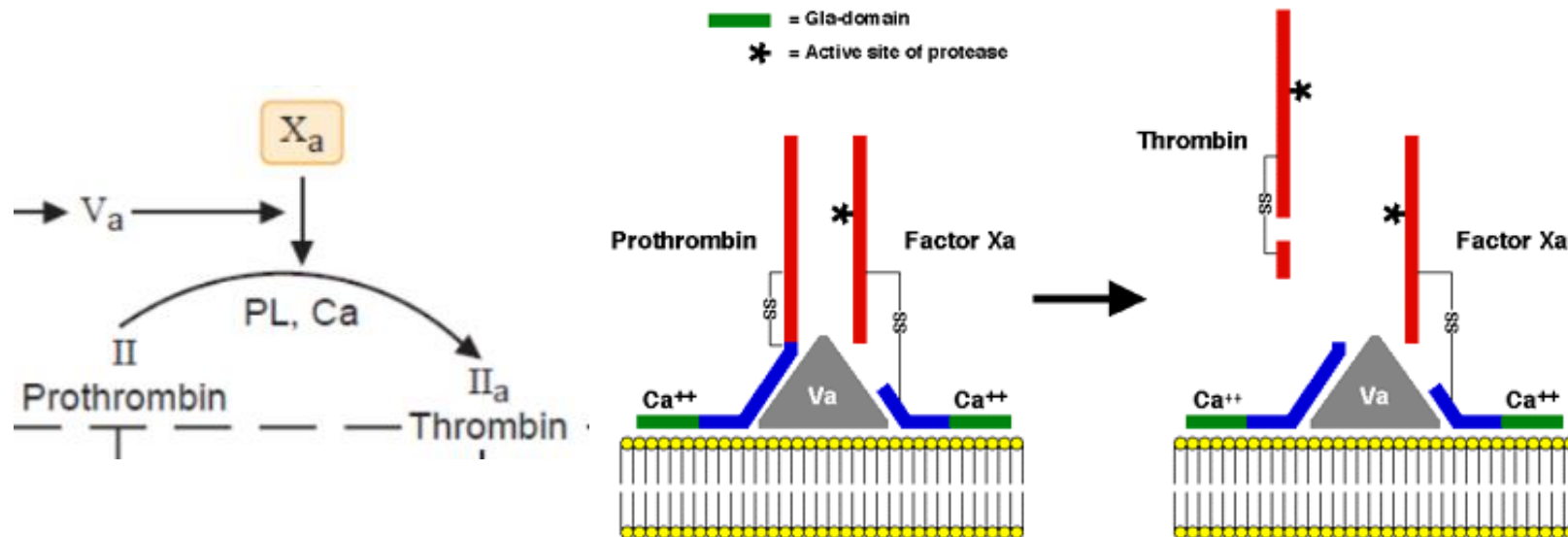
- The activating complexes of factor X are called the “tenase” complexes.
 - The extrinsic tenase complex is made up of tissue factor, factor VIIa, and Ca^{2+} .
 - The intrinsic tenase complex contains the active factor IX (IXa), its cofactor factor VIII (VIIIa), and Ca^{2+} .
 - Tissue factor and factor 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

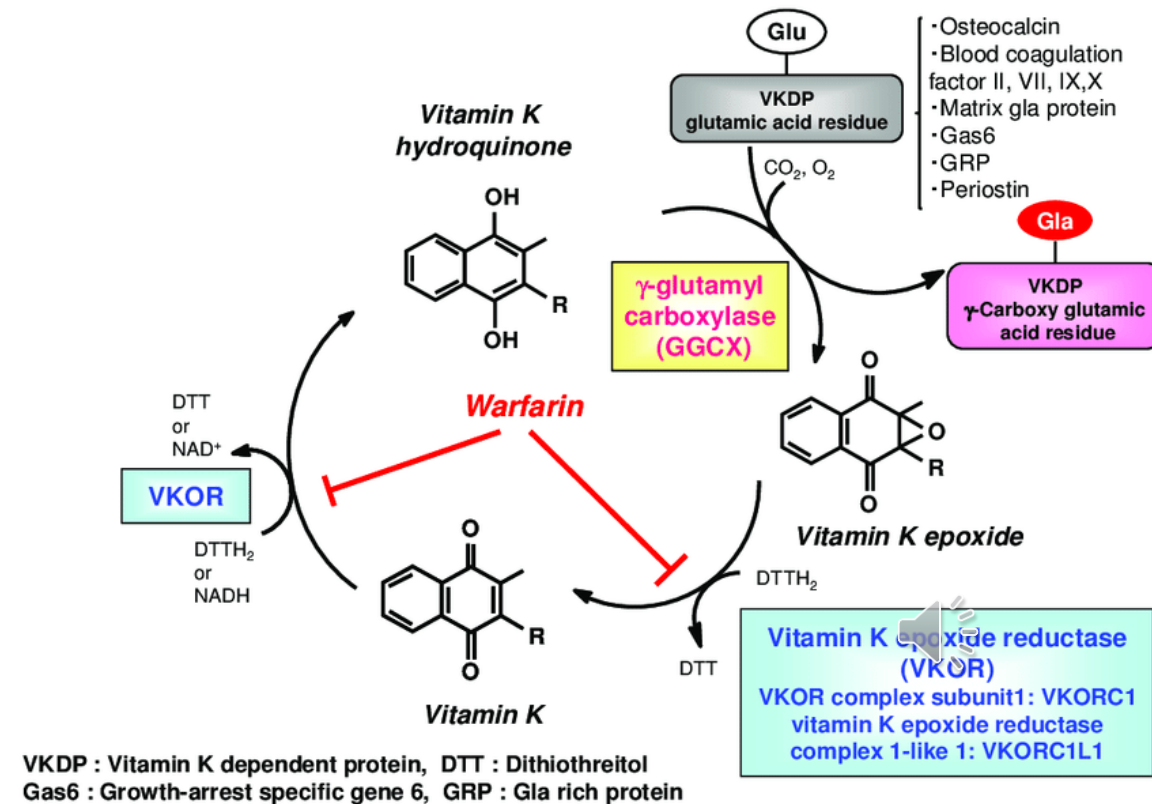
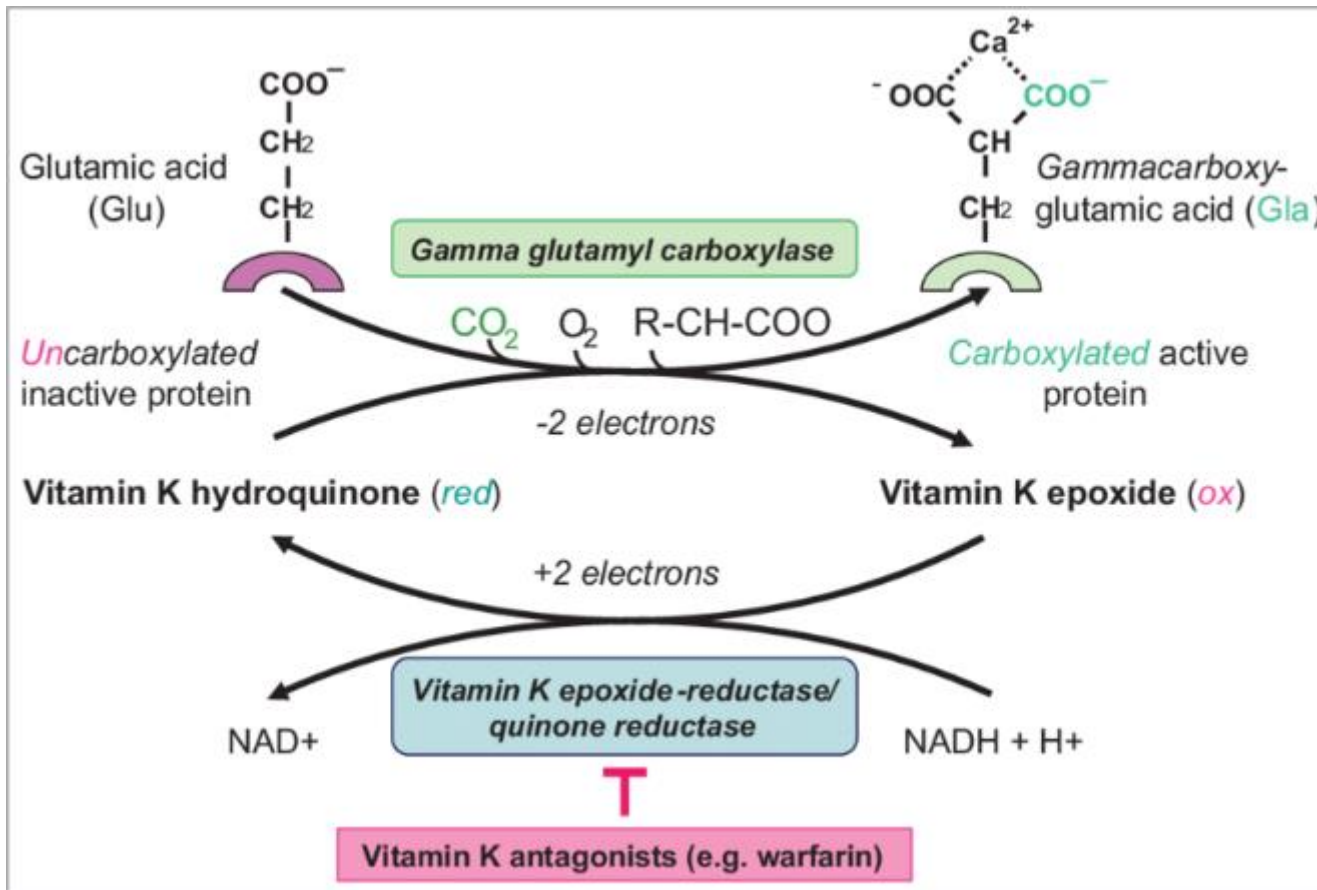
- The complex of factor Xa/Va/Ca²⁺ is the “prothrombinase complex”.
- Factor Xa converts prothrombin to thrombin, which is accelerated by Va, platelets (or phospholipids), and calcium ions.
- Binding of calcium alters the conformation the Gla domains of these factors, enabling them to interact with a 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.



VKDP : Vitamin K dependent protein, DTT : Dithiothreitol
Gas6 : Growth-arrest specific gene 6, GRP : Gla rich protein



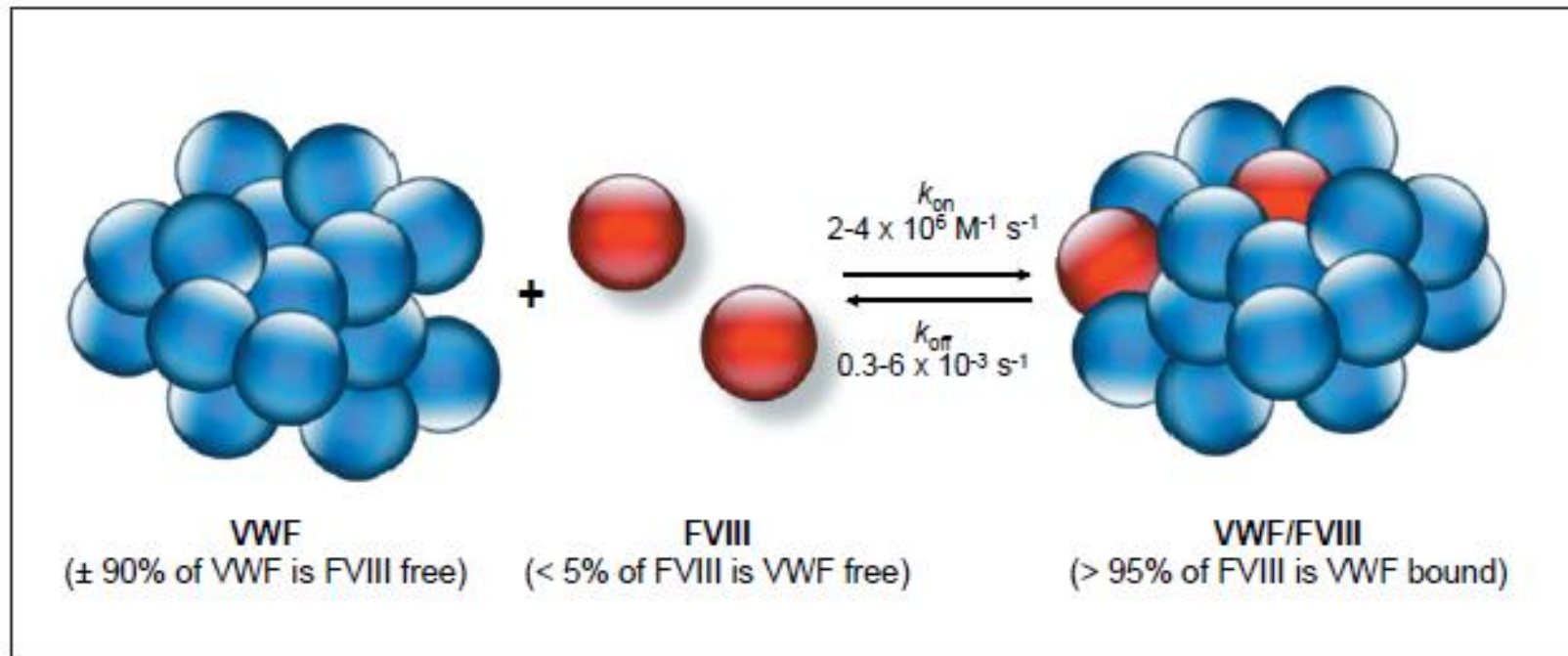
Newborns and vitamin K deficiency

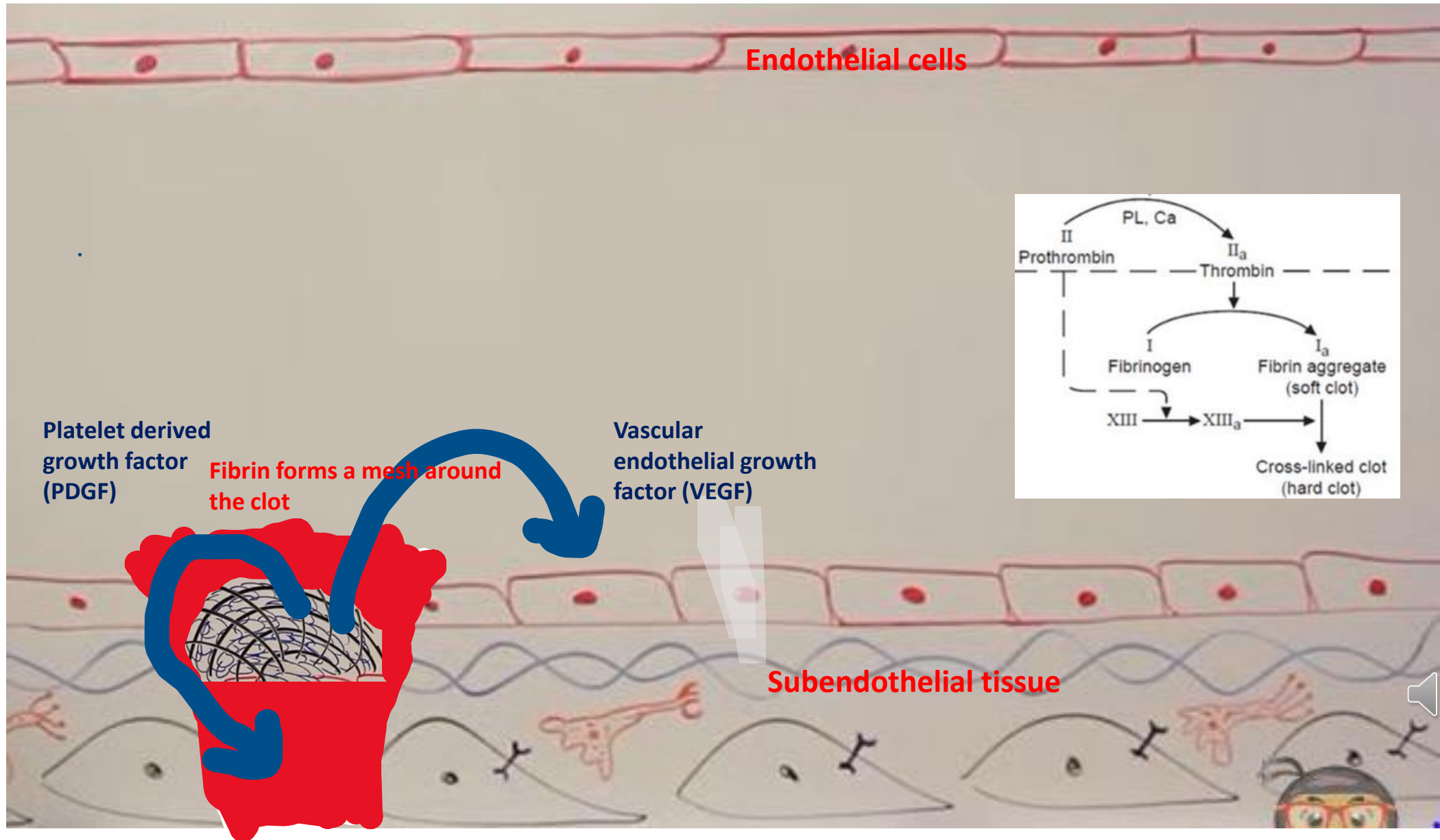
- Newborns are at risk for early vitamin K deficiency bleeding. Why?
 - The placenta is a poor passage channel for fat-soluble compounds, including vitamin K.
 - Neonates are born with an immature liver that impairs coagulation factor synthesis and GLA modifications.
 - Breast milk is a poor source of 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.

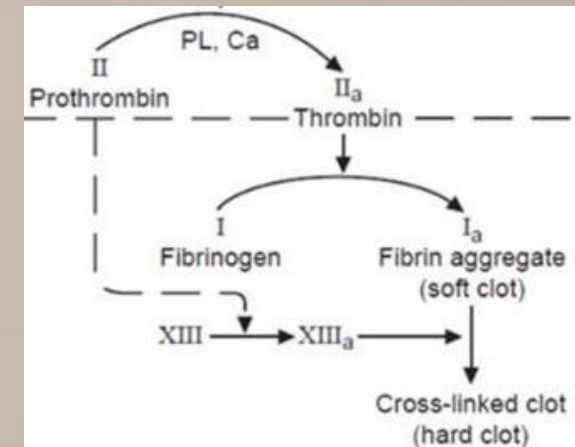






Endothelial cells

- 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.

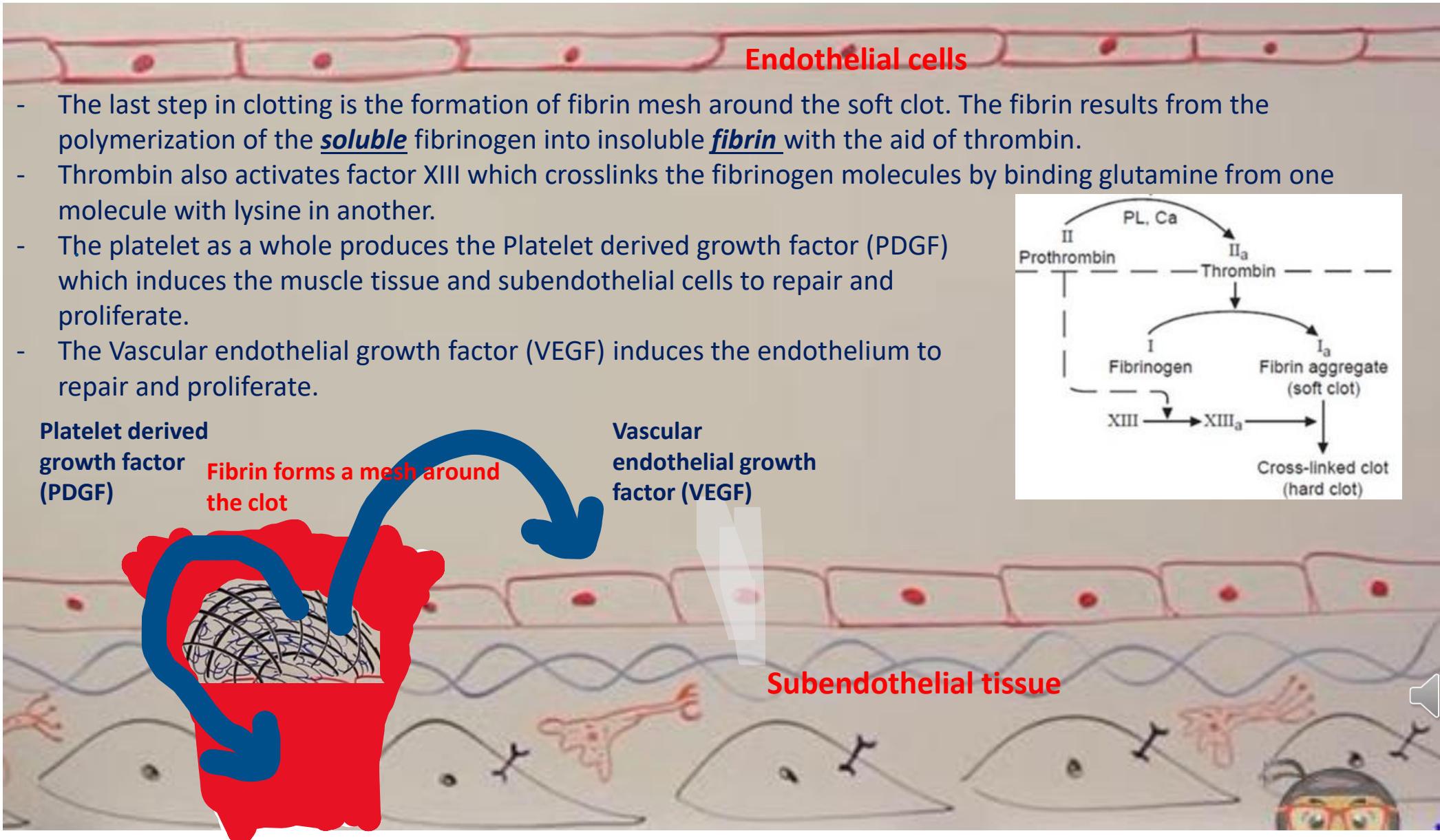


Platelet derived
growth factor
(PDGF)

Fibrin forms a mesh around
the clot

Vascular
endothelial growth
factor (VEGF)

Subendothelial tissue

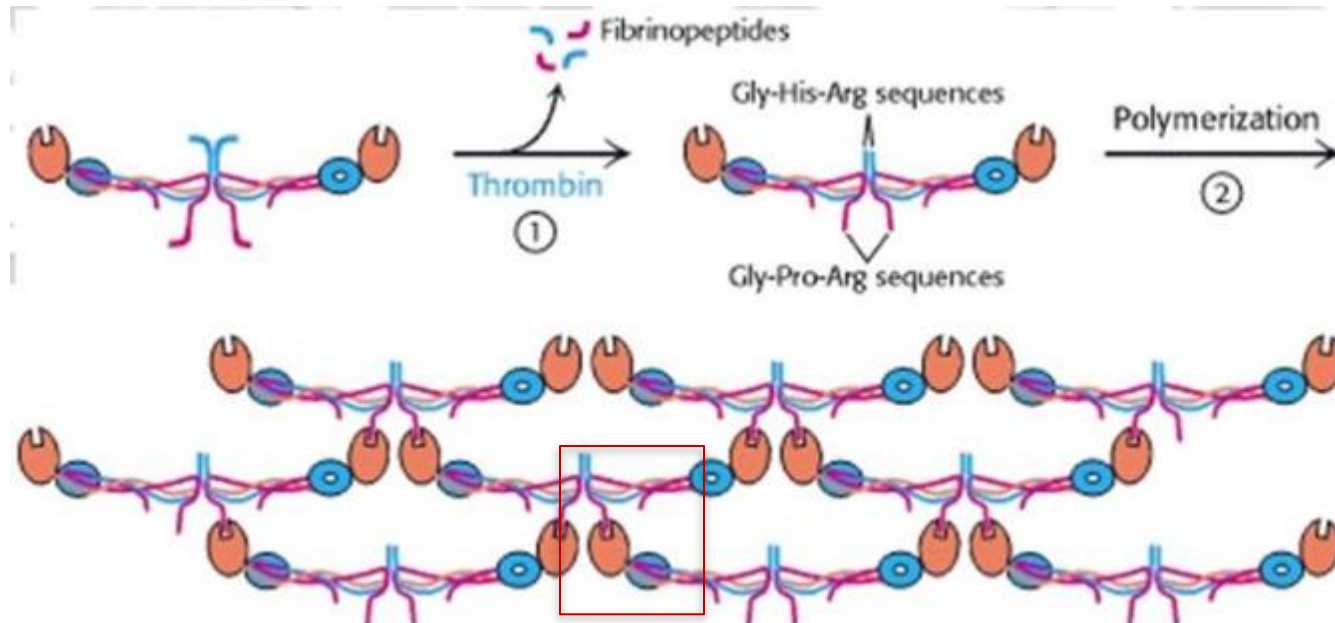




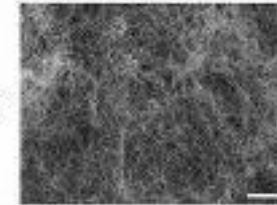
Formation of a *soft* fibrin clot



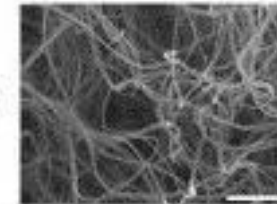
- Thrombin cleaves fibrinogen releasing fibrinopeptides.
 - Fibrinogen is a two triple-stranded helical protein held together by disulfide bonds.
- Fibrin molecules create electrostatic attractions among each other facilitating the aggregation of the monomers into a gel consisting of long polymers.
-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



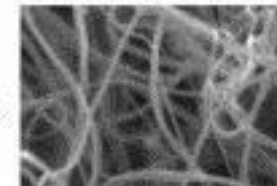
1320X



10900X



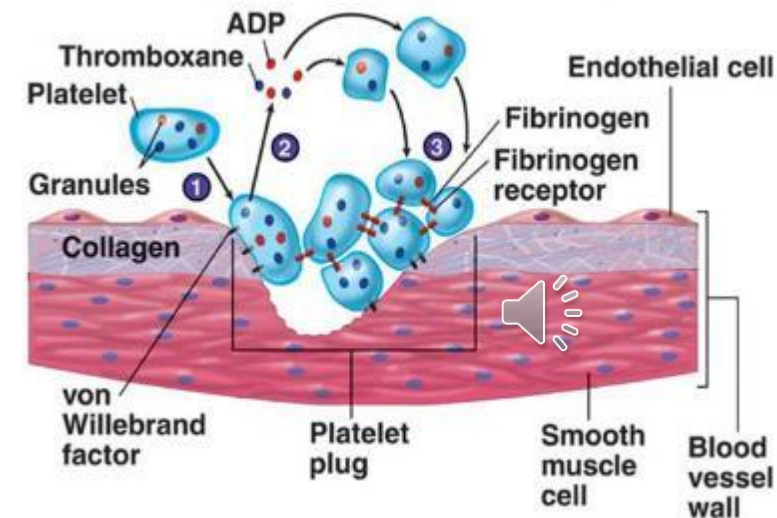
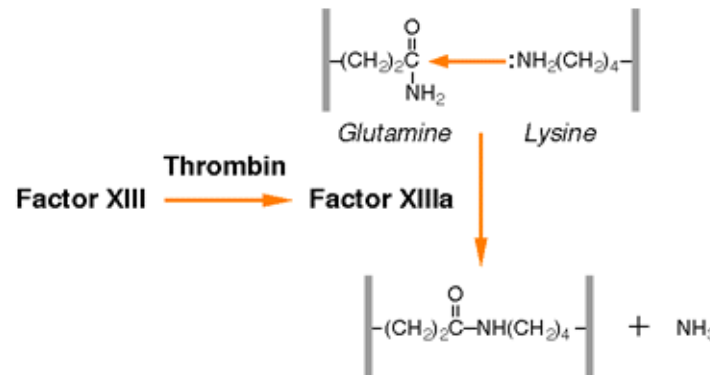
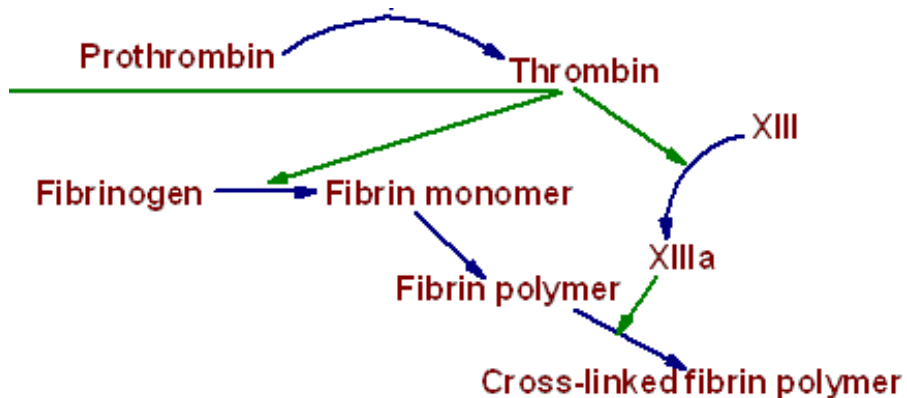
21600X





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.

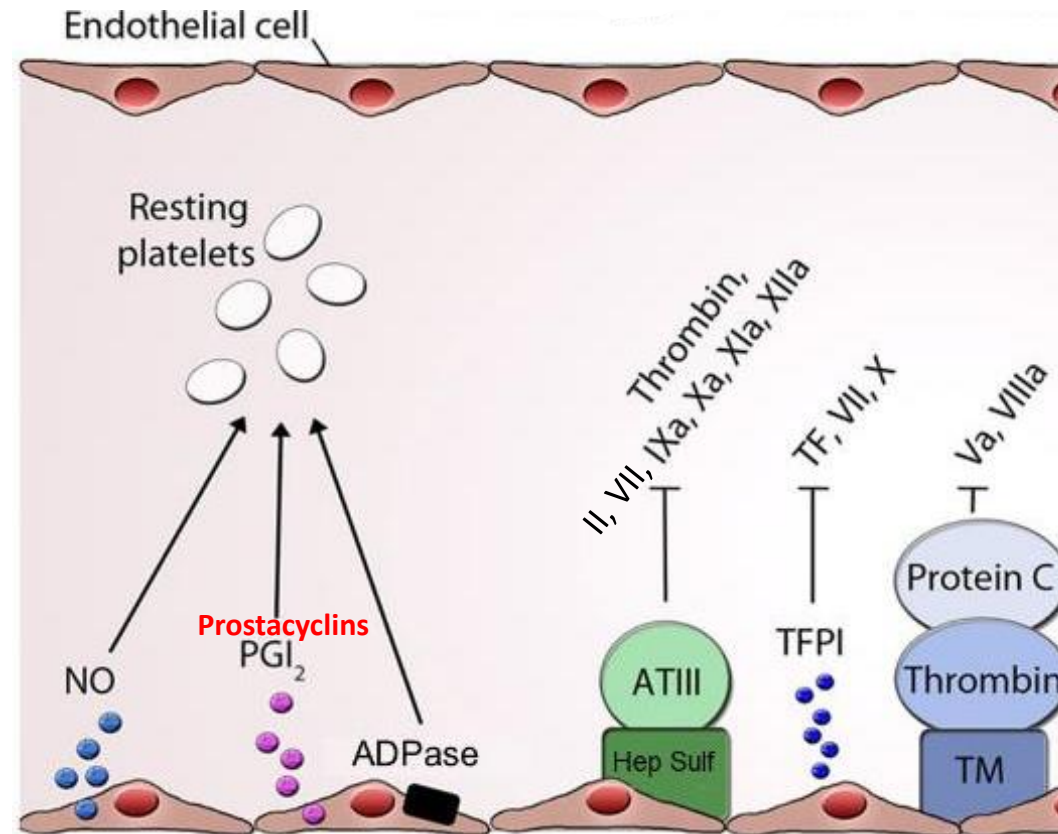




Anti-clotting factors



Role of endothelial cells in coagulation

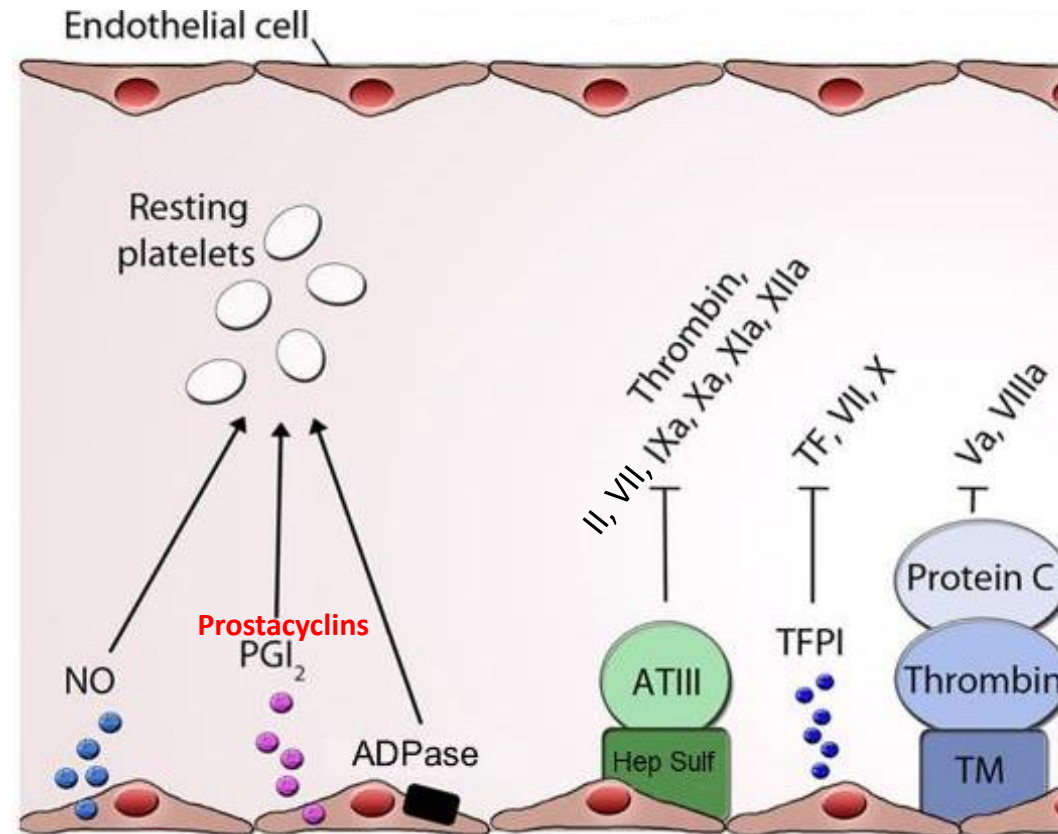


- ECs release NO, prostacyclin (PGI₂), and ADPase, which inhibit platelet adhesion and aggregation.
- Membrane-bound heparin sulfate binds to antithrombin III (ATIII) inactivating several coagulation factors.
- ECs express tissue factor pathway inhibitor (TFPI), which inhibits tissue factor (TF) and,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





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.
- Ca²⁺ Chelators such as **EDTA** (**not used for humans, only for phlebotomy tubes**)

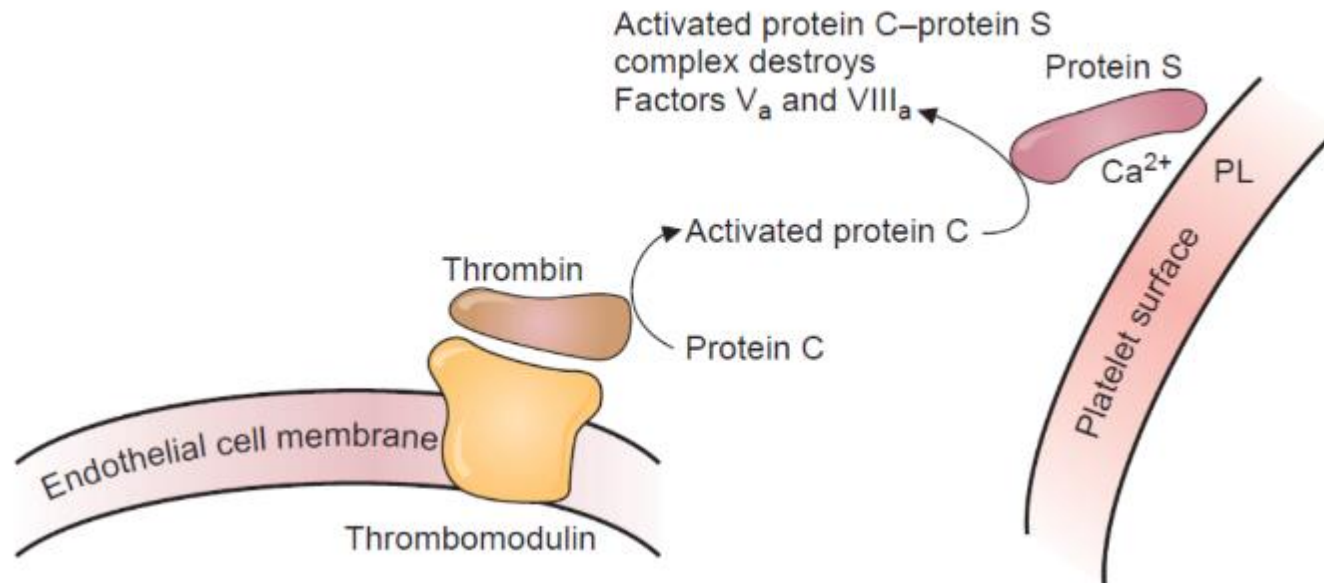
- ECs release NO, prostacyclin (PGI₂), and ADPase, which inhibit platelet adhesion and aggregation.
- Membrane-bound heparin sulfate binds to antithrombin III (ATIII) inactivating several coagulation factors.
- ECs express tissue factor pathway inhibitor (TFPI), which inhibits tissue factor (TF) and,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





Protein C and protein S

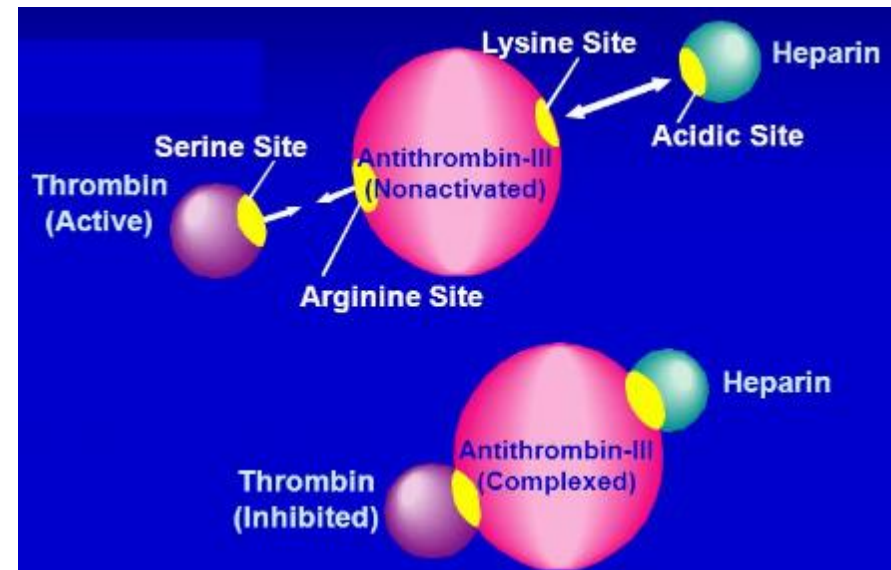
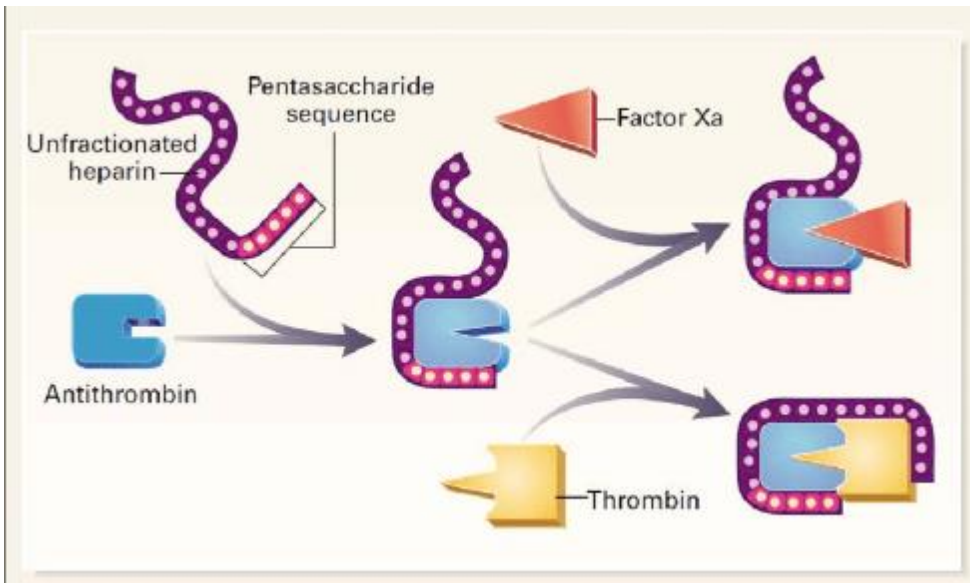
- Thrombin binds thrombomodulin on the surface of endothelial cells.
- Thrombin can then activate protein C, which forms a complex with protein S, both of which are vitamin K-dependent cofactors.
- The complex degrades factors V and VIII.





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.

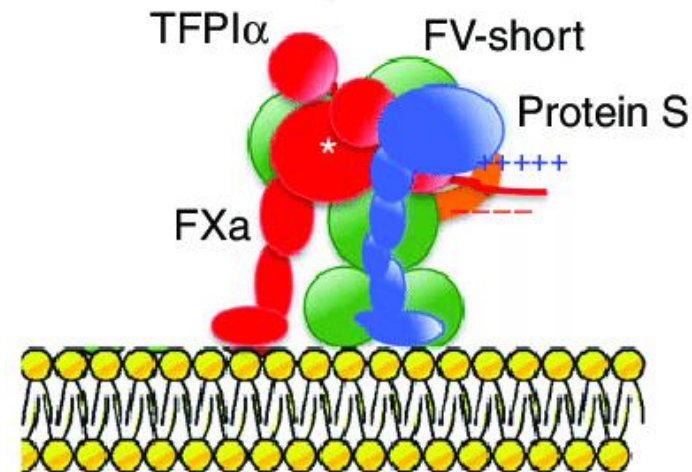
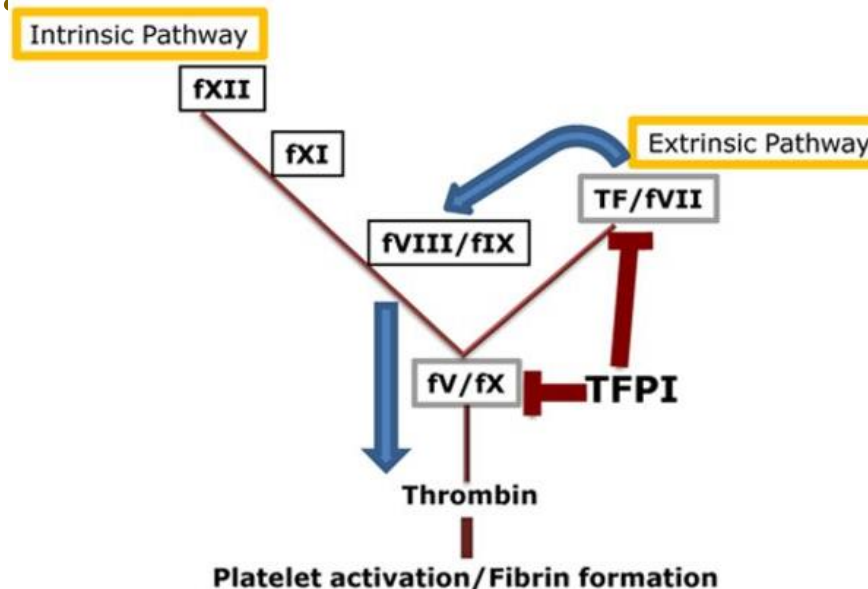


In the clinic, phlebotomy tubes are often treated with heparin to inhibit clot formation.



Tissue Factor pathway inhibitor

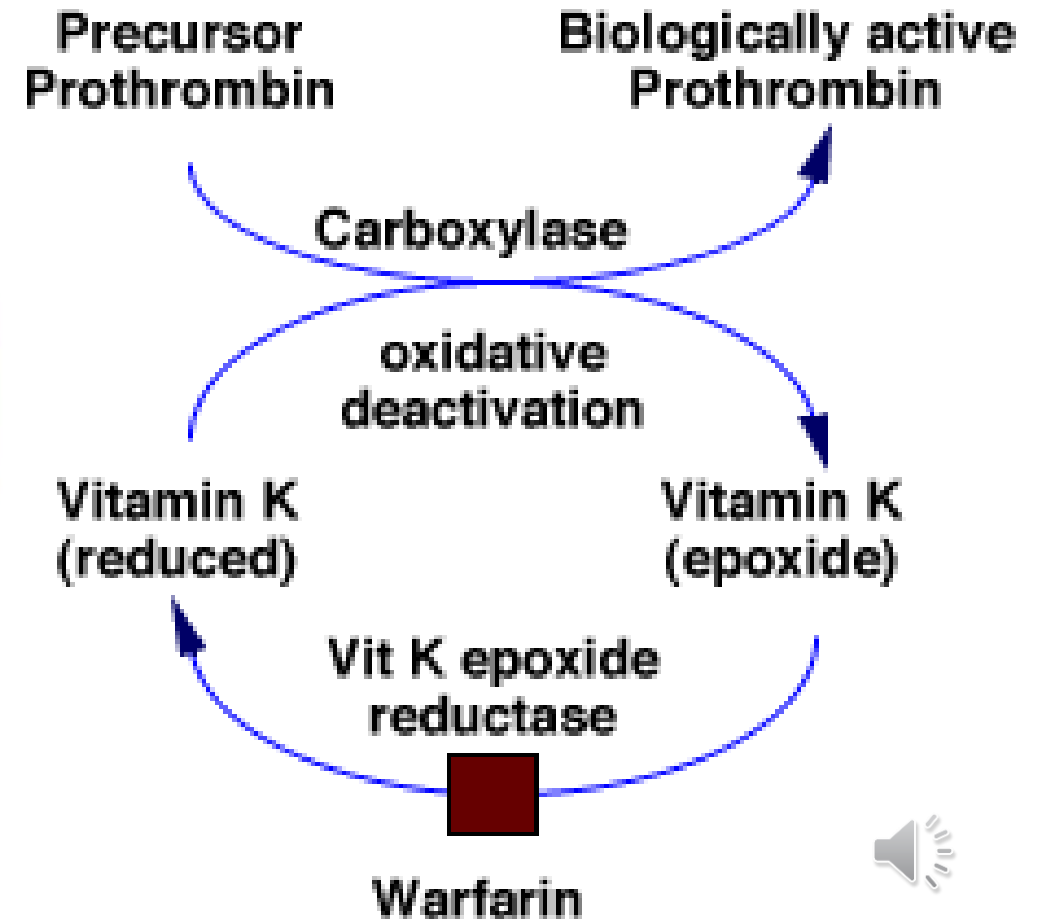
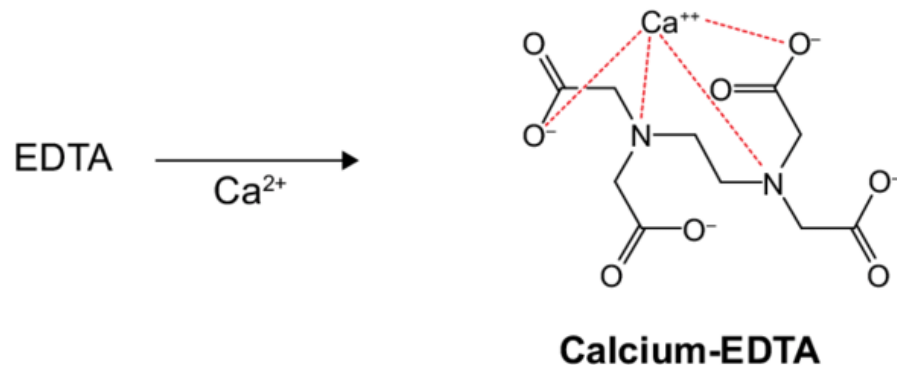
- Tissue factor pathway inhibitor (TFPI) is a protein found in plasma lipoproteins and bound to the vascular endothelium.
 - The Xa-TFPI complex then interacts with the TF-VIIa complex and inhibits its activation of factors X and IX.
 - TFPI also inhibits Xa-activated Va resulting in inhibition of the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





Ca²⁺ chelators and vitamin K antagonists

- Blood clotting can be prevented by addition of Ca²⁺ 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

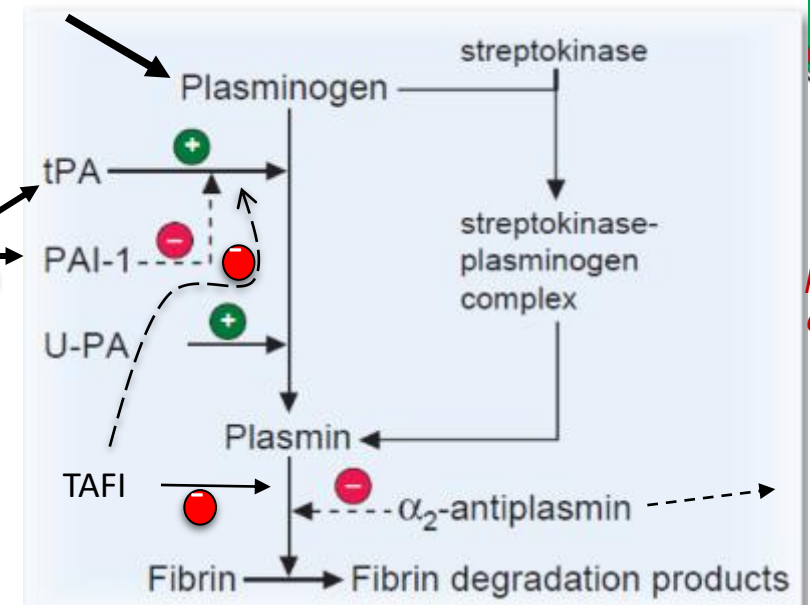
- Factor XII and Thrombin activate Plasminogen through the tissue plasminogen activator tPA. tPA is the main player in the fibrinolysis. Plasmin is like trypsin it cuts proteins at certain sequences, and its activity is highly increased upon binding to fibrin as it puts it in proximity to plasminogen.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

Thrombin

Releases

Activated
protein C

Factor XII



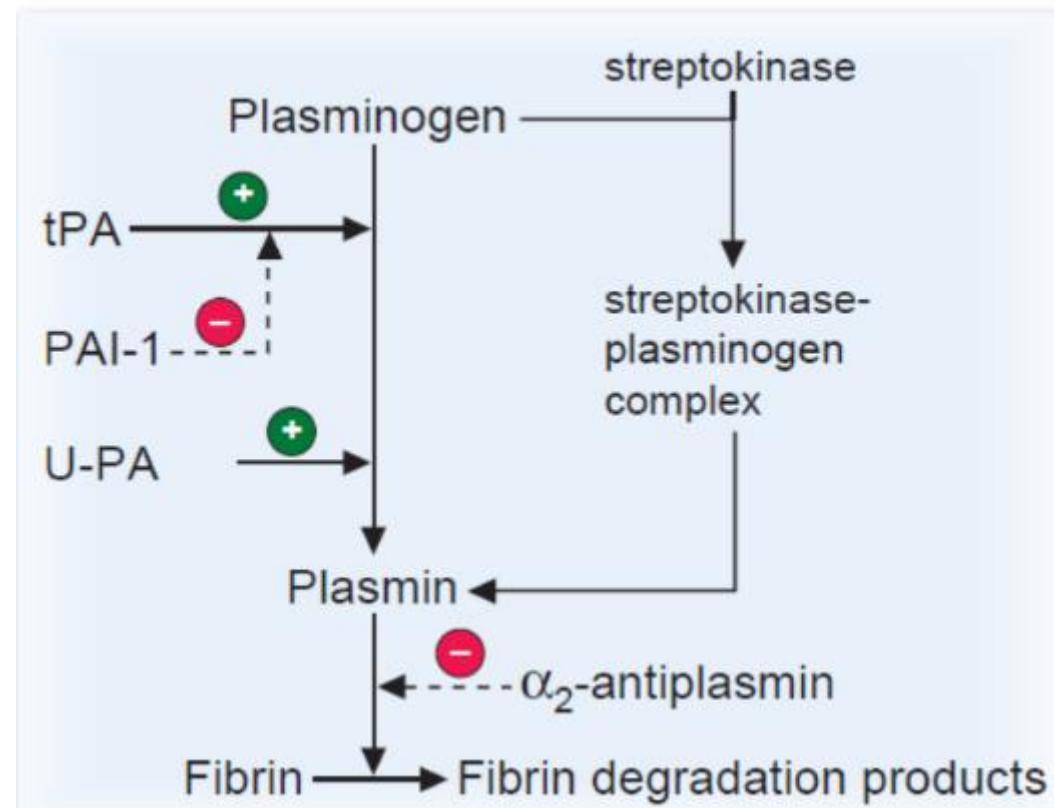
but not
when
plasmin
ogen/pl
asmin
are
clot-
bound





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.

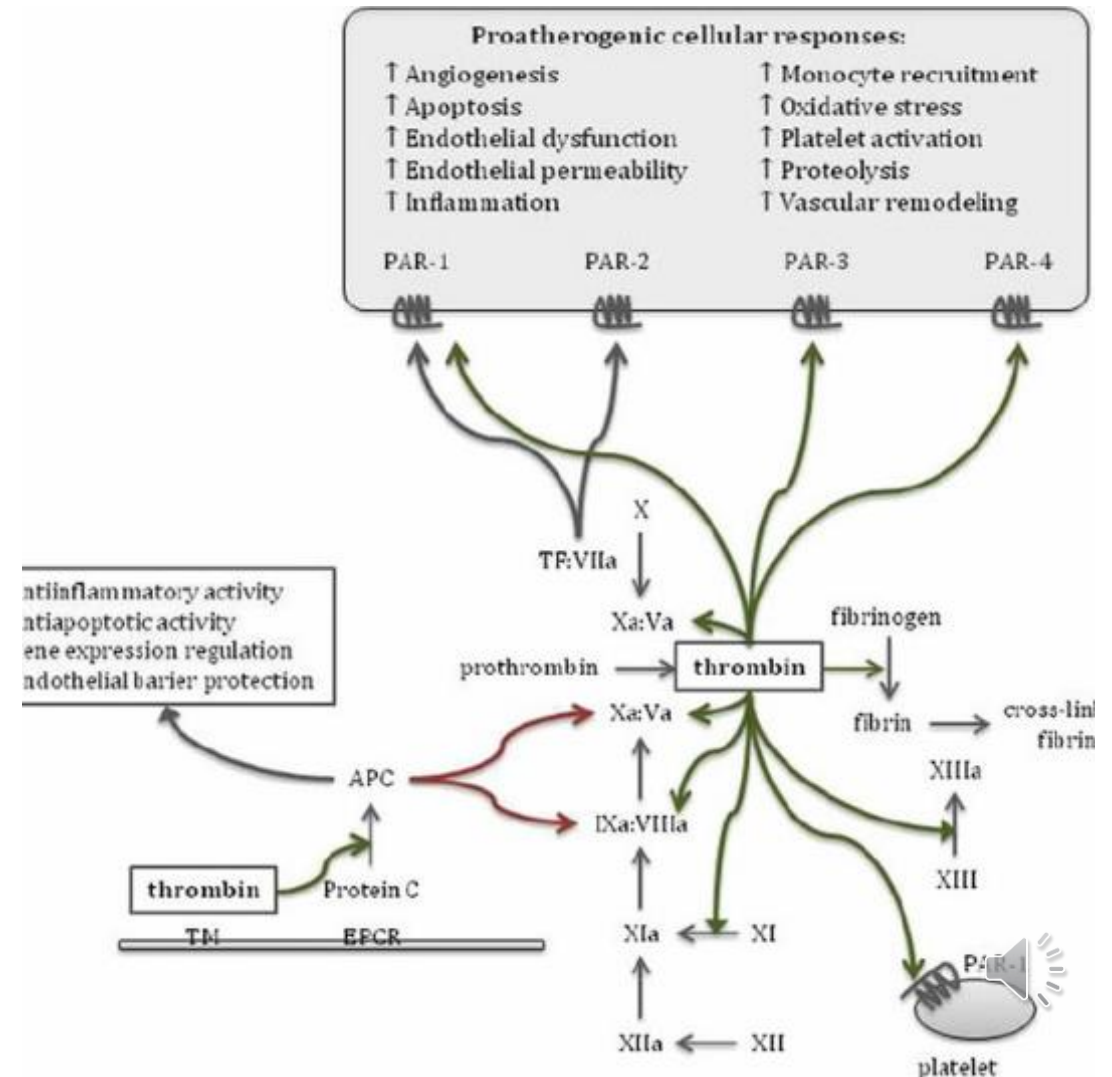




Table 3: illustrates the engagement and detailed explanation of coagulation factors, that aid in the blood coagulation cascade

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	Proaccerein / 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 factor VIII)
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 fibrinopeptides allowing a mesh to form around the platelets. The mesh is not very strong yet. It is further strengthened 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.

