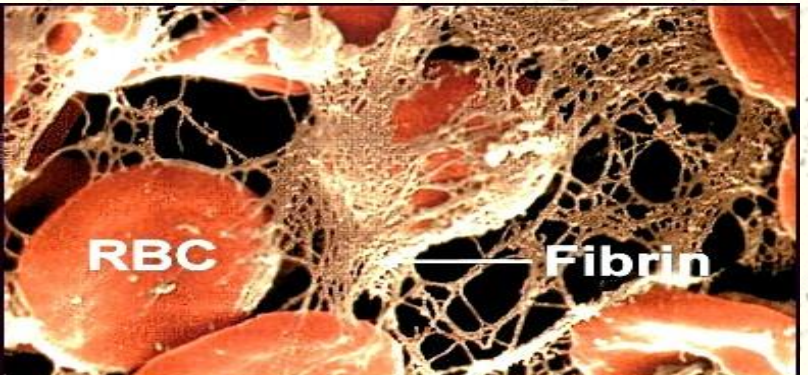
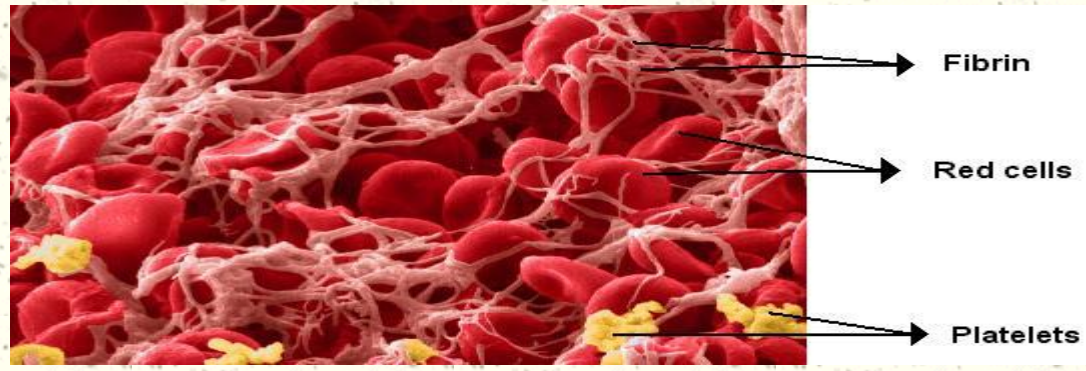
Hemostasis and Blood Coagulation

Dr.TAHANI ALI





Objectives

- By the end of this lecture the student should be able to:
  - Define haemostasis.
  - Describe the main mechanisms that prevent blood loss after an injury.
  - Describe role of platelets in haemostasis
  - Outline the mechanism of platelet plug formation.
  - Describe the mechanisms of blood coagulation

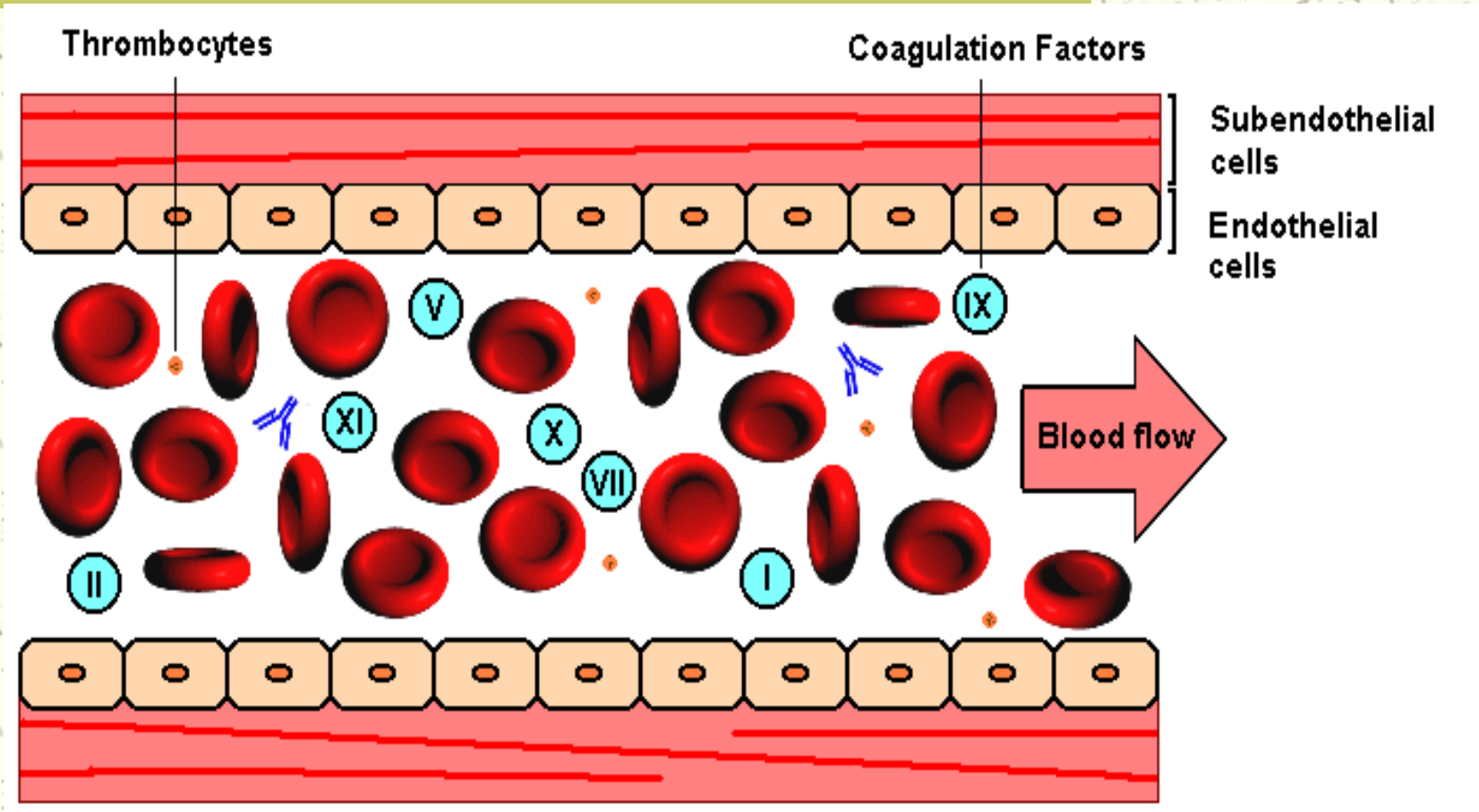
Haemostasis

* Hemostasis: drives from the Greek meaning "The stoppage of blood flow".
* The term *haemostasis* means prevention of blood loss.
* Haemostasis is the process of forming clots in the walls of damaged blood vessels and preventing blood loss, while maintaining blood in a fluid state within the vascular system.

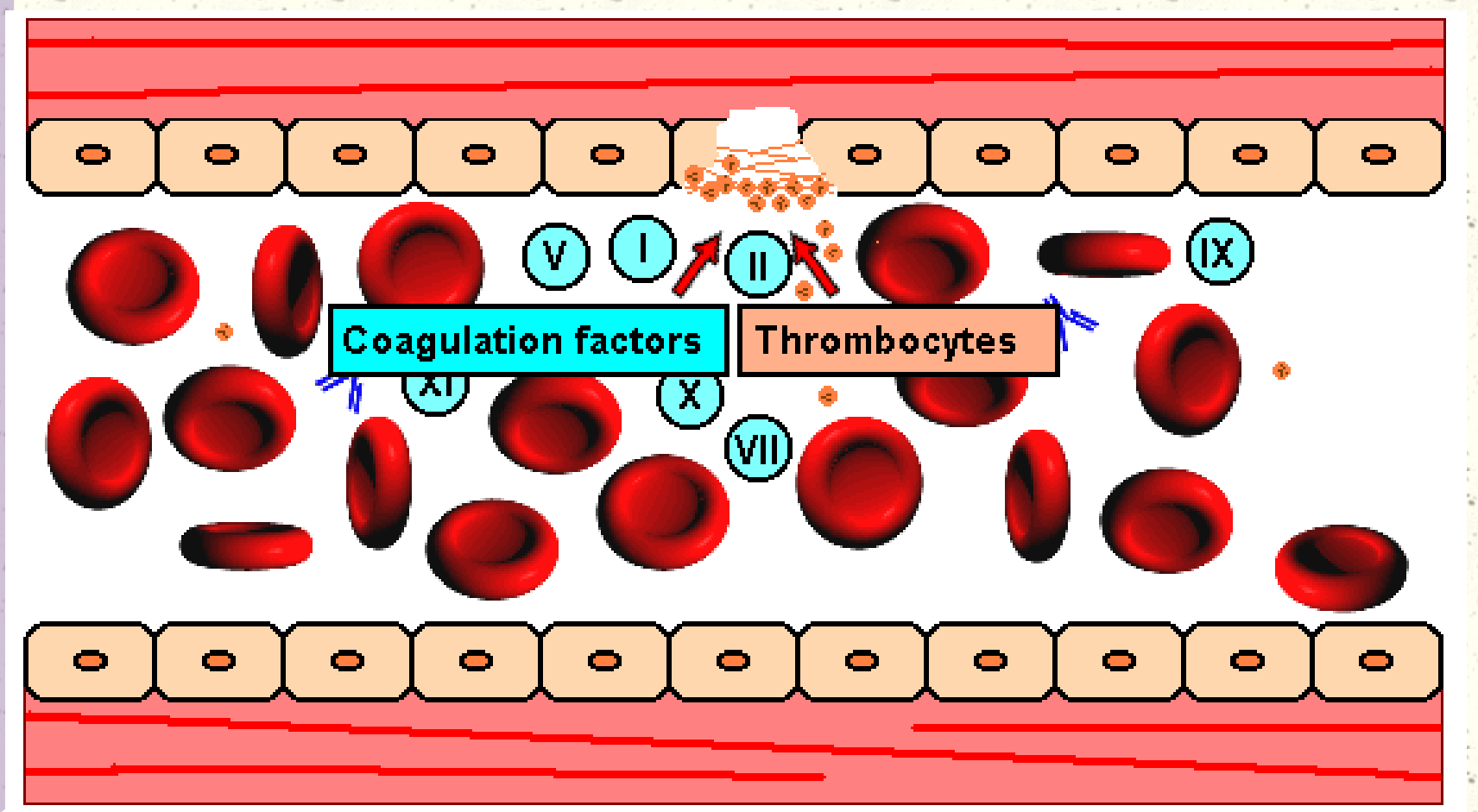
Definition of HEMOSTASIS

- Maintaining a balance
  - Coagulation
  - Fibrinolysis
- Hypocoagulation: excessive bleeding (inherited or acquired)
- Hypercoagulation (thrombosis) inadequate activation of the fibrinolytic system

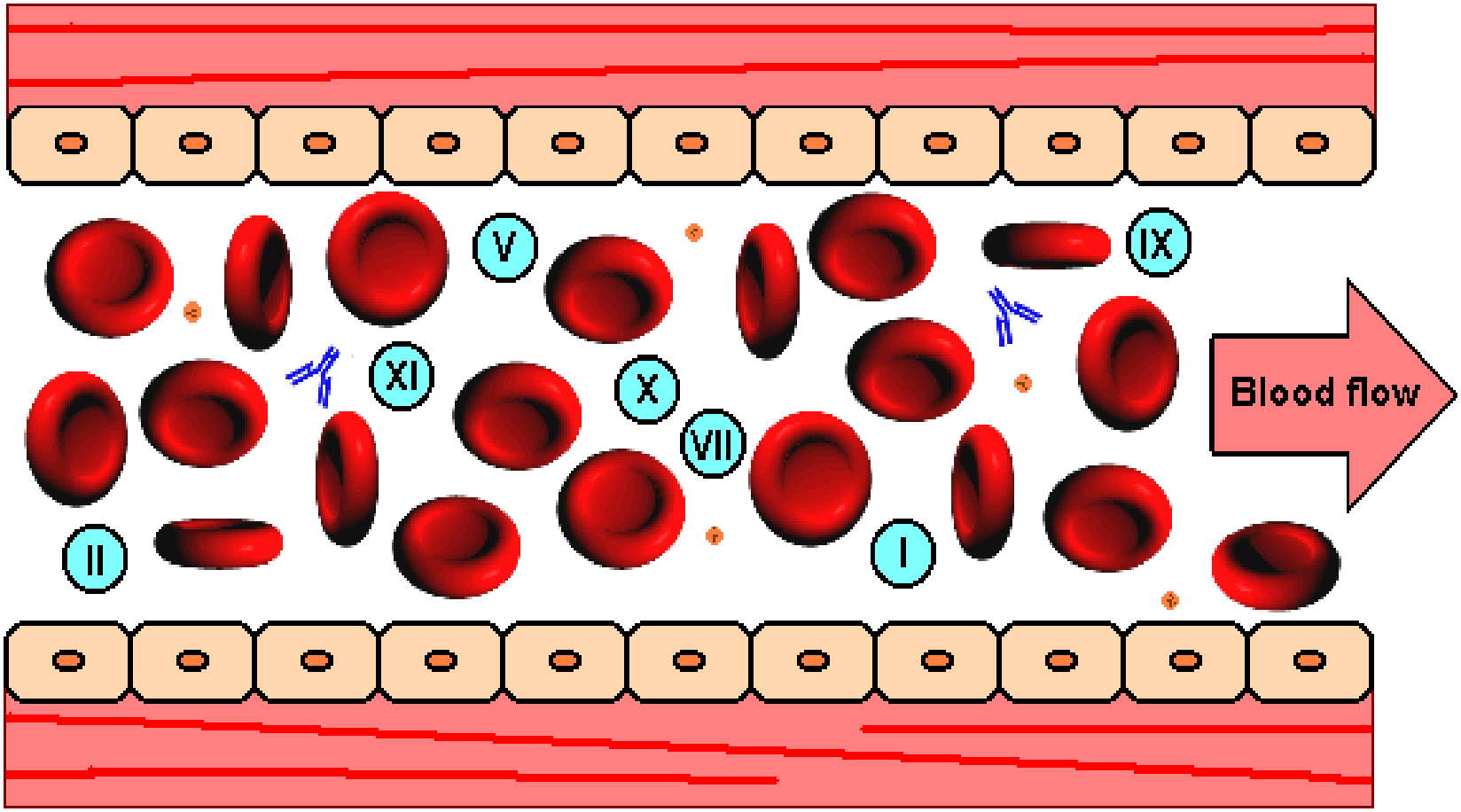
Vessel wall, Blood flow & Coagulation Substances



In Case if there is an Endothelial Injury (Bleeding must be prevented at site of injury)



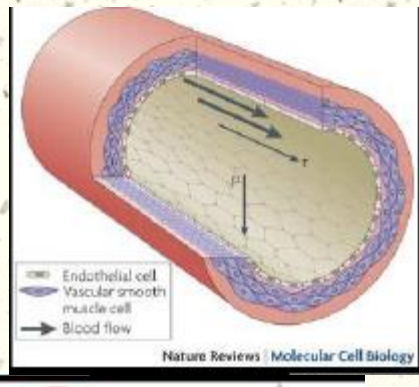
Flow must be Maintained

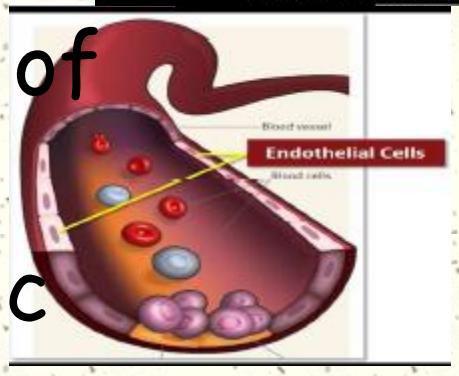


Systems Involved in Hemostasis

- **Vascular system**
  - Injured vessel initiates vasoconstriction
- **Platelet System**
  - Injured vessel exposes collagen that initiates platelet aggregation and help form plug
- **Coagulation System**
  - protein factors of intrinsic and extrinsic pathways produce a permanent fibrin plug

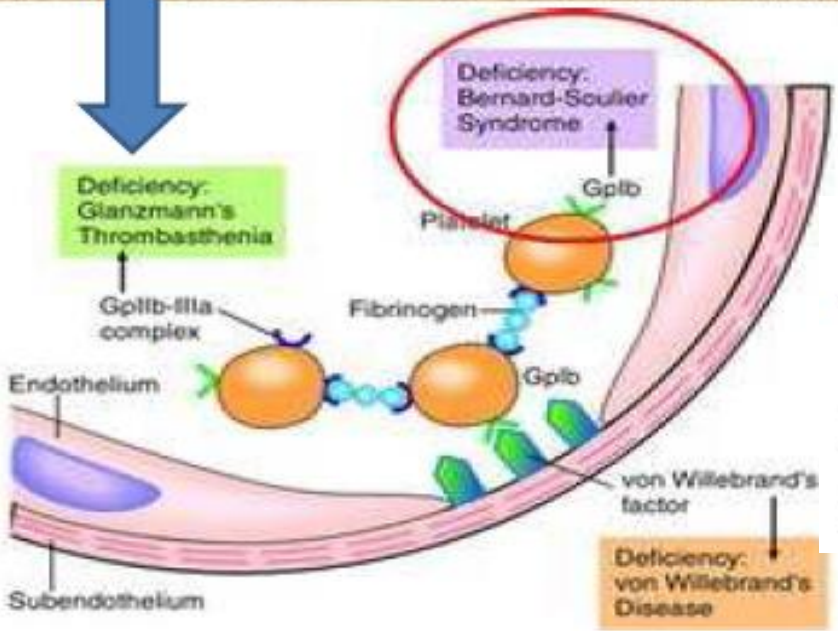
Endothelium





- Key players in the regulation of homeostasis
- Have Anti- and prothrombotic activities:
  - Thrombus formation
  - Propagation, or
  - Dissolution occurs

Endothelium



- Normal endothelial cells exhibit

Antithrombotic activity:

1. **Antiplatelet**
2. **Anticoagulant and**
3. **Fibrinolytic properties**

In summary

- **Intact, nonactivated** endothelial cells ⇨ **Antithrombotic**
- **Endothelial injury or activation** ⇨ **Prothrombotic**

Platelets

- Fragments of *megakaryocytes*; diameter 1-4 µm, 150-400x10^9 /l of blood
- Contain many active factors:
  - Actin, myosin, thrombosthenin
  - Endoplasmic reticulum, Golgi apparatus
  - Mitochondria
  - Enzymes for prostaglandin production
  - Fibrin-stabilizing factor
  - Growth factors for vascular repair
  - Glycoproteins on cell surface

Functional characteristics of platelets

The cell membrane of platelets contains:

- A coat of **glycoprotein** (receptors) that cause adherence to injured endothelial cells and exposed collagen:
  - Ib (GPIb)
    - Receptor site for vWF
  - IIb, IIIa (GPIIb/IIIa)
  - Complex becomes receptor site for fibrinogen
- **Phospholipids**, that play an important role in blood clotting

Granular content

- Dense granules
  - ATP
  - ADP
  - **Calcium**
  - Magnesium
  - **Serotonin**
  - epinephrine

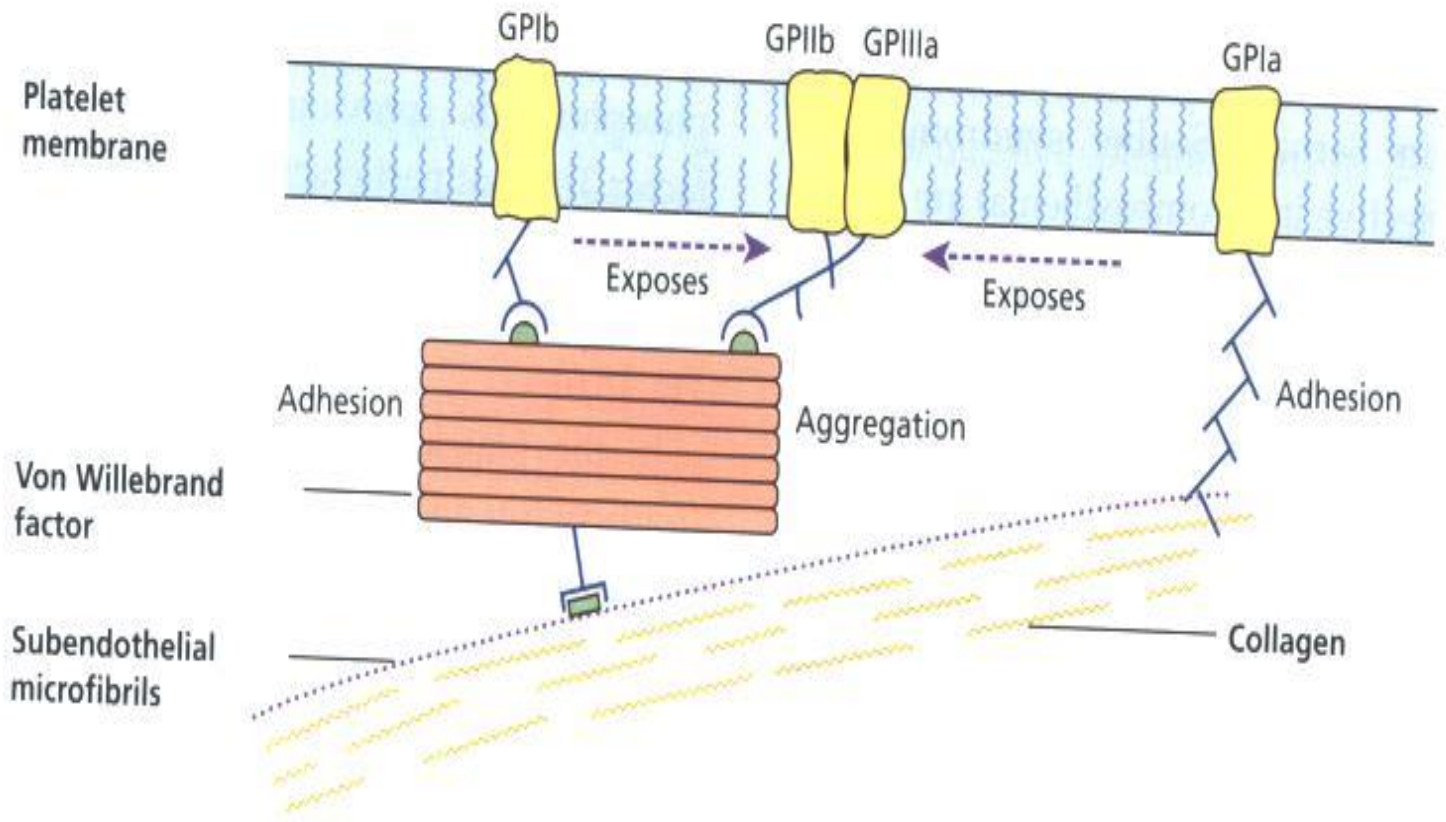
Granular content (Alpha granules)

- Hemostatic proteins
  - **Fibrinogen**
  - **Factor V**
  - **vWF**
  - Plasminogen
  - Plasminogen activator inhibitor (PAI-1)
  - α_2-antiplasmin
- Nonhemostatic proteins
  - β-thromboglobulin,
  - Platelet factor 4
  - Platelet derived growth factor (PDGF)
  - Albumin
  - fibronectin,

VON WILLEBRAND FACTOR

- Large Adhesive Glycoprotein
- Polypeptide chain: 220,000 MW
- Base structure: Dimer; Can have as many as 20 linked dimers
- Multimers linked by disulfide bridges
- Synthesized in endothelial cells & megakaryocytes
- Constitutive & stimulated secretion
- Large multimers stored in Weibel-Palade bodies
- Functions:
  1) Stabilizes Factor VIII
  2) Essential for platelet adhesion

VON WILLEBRAND FACTOR



Classification of Coagulation Factors

Based on functional or structural properties

- Physical groupings
  - Prothrombin
  - Fibrinogen
  - Contact
- Functional Groupings
  - Substrate
  - Cofactors
  - Enzyme

Physical Groupings

Prothrombin Group

- II, VII, IX, X
- Protein C, Protein S, Protein Z
- Vitamin K dependent
- Synthesized in liver
- Small mw (50,000-100,000)
- Contain a domain that is critical for calcium binding
- Heat stable
- Inhibited by warfarin

Fibrinogen group

- I, V, VIII, XIII
- Thrombin acts on all these factors
- Synthesized in liver
  - Exception: VIII:vWF which is produced by endothelial cells and megakaryocytes
- Large mw (250,000)
- ALL are consumed in the clotting process, since they are NOT enzymes

Physical Groupings

Contact Group

- XI, XII, HMWK(HK), PK
- Produced in liver
- Activated upon ~~s~~**contact** with a negatively charged surface
  - Collagen ~~in v~~*in vivo*
  - Glass, Kaolin *in vitro*
- Large mw (80,000-173,000)
- Not consumed in coagulation, found in serum
  - Purpose: activate the **intrinsic** pathway & fibrinolytic system

Functional Groupings

- **Substrates**: substance upon which enzymes act
  - Factor I:fibrinogen
- **Cofactors**: speed up the activities of enzymes
  - (i.e) Factor V: Proaccelerin
- **Enzymes**
  - Transglutaminase
    - Factor XIIIa only
  - Serine protease
    - Inactive until converted to enzymes
    - Once activated, assist in reaction, but are not consumed or used up

What's so Special About Vitamin K?

- Where does it come from?
  - Green leafy vegetables, fish and liver
  - Gram-negative intestinal bacteria
- What does it do?
  - Vitamin K is necessary for the carboxylation of glutamic acid. Carboxylation is essential for binding coagulation factors to negatively-charged phospholipid surfaces via Ca^{++} bridges. Carboxylation reactions also reduce vitamin K to be recycled.

What's so Special About Vitamin K?

- Why do we care?
  - Vitamin K antagonist drugs such as warfin/coumadin inhibit the activity of the recycling of Vitamin K, so the reduced form can not be made
  - Deficiencies of Vitamin K result in the production of non-functional factors which can not participate in coagulation reactions

Coag factors (by group)

- **Fibrinogen group**: I,V,VIII,XIII
  - most labile, are consumed in coagulation, found on platelets
- **Prothrombin group**: II,VII,IX,X
  - Vitamin K dependent, may be affected by coumarin,diet, antibiotics
- **Contact group**: XI,XII,HMWK, Prekallikrein
  - initiate intrinsic path and fibrinolysis

agulation Proteins

- we don't really call it factor III anymore. we know now that it is a specific protein called tissue factor

Factor	Synonyms	Function
I	Fibrinogen	polymer unit
II	Prothrombin	protease
III	Tissue thromboplastin, tissue factor	cofactor
IV	Calcium	
V	Accelerator globulin, proaccelerin, labile factor	cofactor
VII	Proconvertin, stable factor	protease
VIII	Antihemophilic factor or globulin	cofactor

we don't call Calcium factor IV either...

released from platelets in a partially active form, which was known as factor VI, but we don't call it that

Factor	Synonyms	Function
IX	Christmas factor, plasma thromboplastin component	protease
X	Stuart factor, Stuart-Prower factor	protease
XI	Plasma thromboplastin antecedent	protease
XII	Hageman factor	protease
XIII	Fibrin stabilizing factor, fibrinoligase	Fibrin crosslinker
----	Prekallikrein (Fletcher factor)	protease
----	High-molecular-weight kininogen (Fitzgerald factor)	cofactor

unless otherwise specified, we still use the factor name and number to identify parts of the cascade. :)

Factor VI was at one time used to designate activated Factor V.

HEMOSTASIS

Primary vs. Secondary vs. Tertiary

- **Primary Hemostasis**
  - Platelet Plug Formation(Unstable)
  - Dependent on normal platelet number & function
  - Initial Manifestation of Clot Formation
- **Secondary Hemostasis:** Activation of Clotting Cascade
  - ✉ Deposition & Stabilization of Fibrin
  - Reinforced platelet plug with fibrin clot
  - Enzyme-mediated, cascade-like reactions
  - End result = insoluble fibrin clot
- **Tertiary Hemostasis**
  - Dissolution of Fibrin Clot
  - Dependent on Plasminogen Activation

Primary Hemostasis

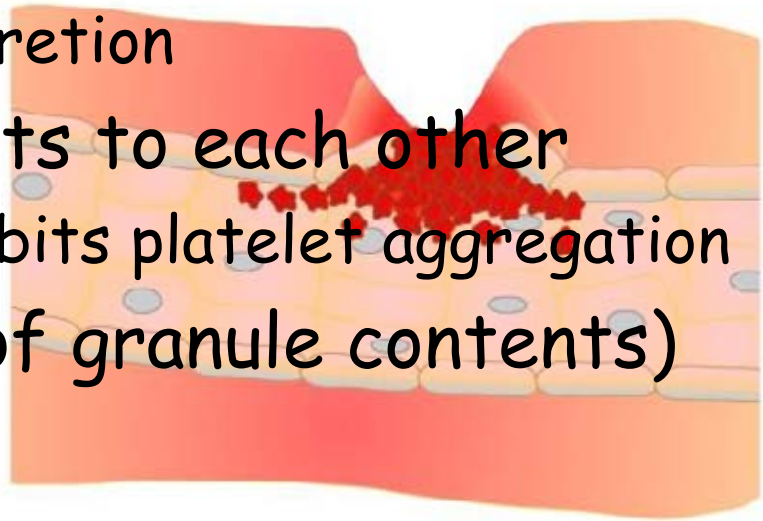
- vasoconstriction (vascular system)
- platelet exposure to sub-endothelial connective tissue of blood vessels
- Platelet release of ADP, ATP, Thromboxane A_2 (promotes vasoconstriction)
- Platelet aggregation, phospholipid provides site for fibrin formation

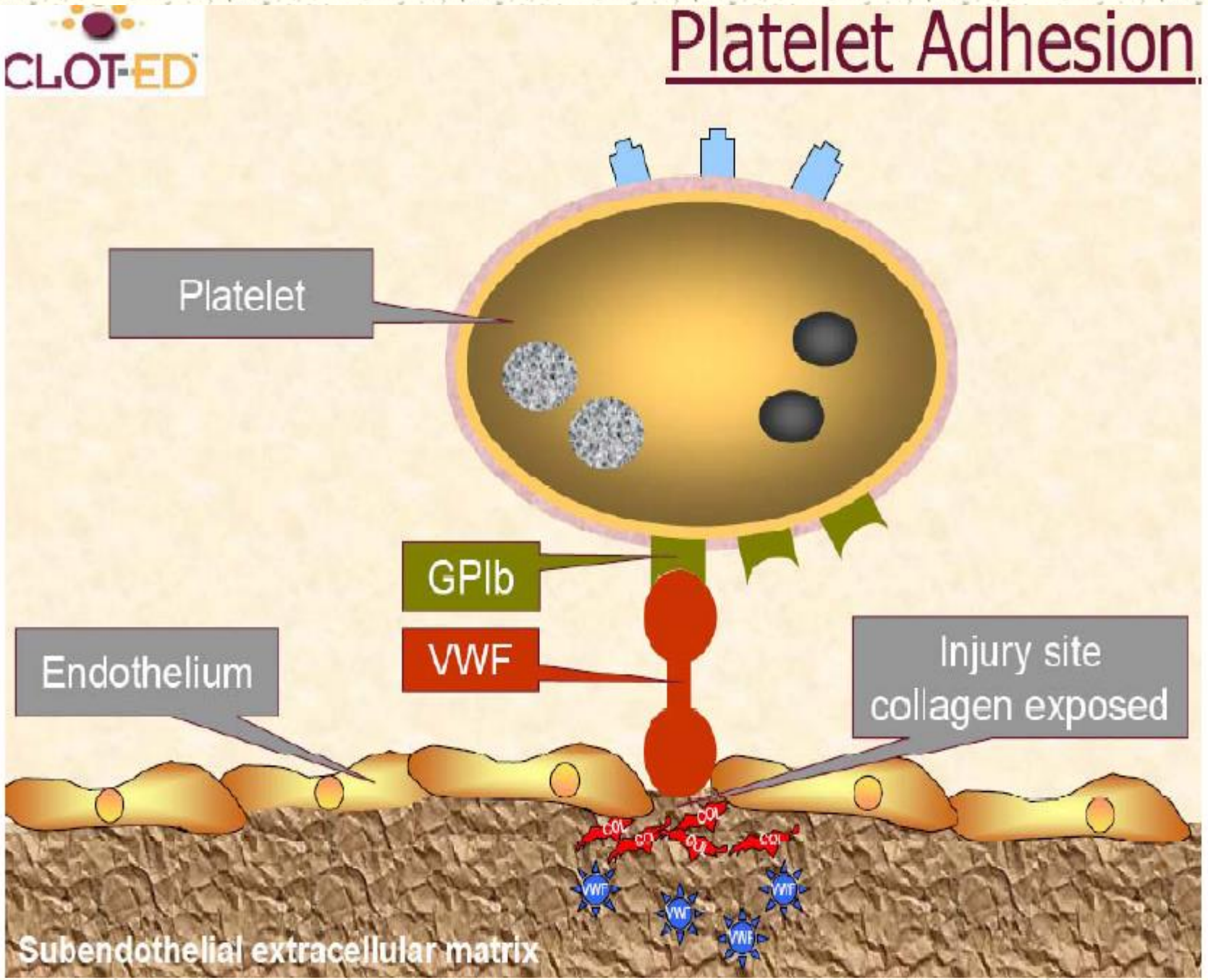
I-Vascular spasm

- Reduces flow of blood from injured vessel.
  - Sympathetic reflex
- Release of vasoconstrictors (TXA_2 and serotonin) from platelets that adhere to the walls of damaged vessels

Formation of primary hemostatic plug

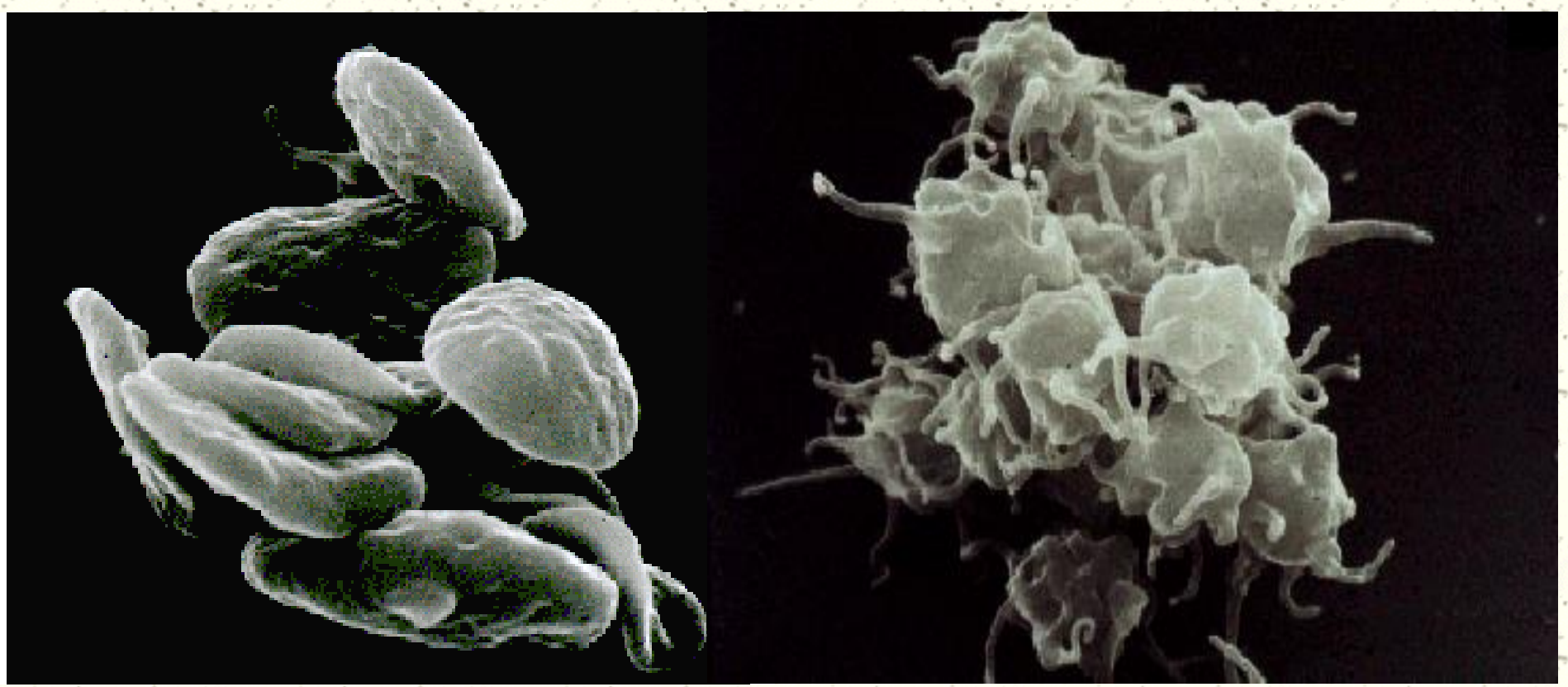
- Platelets converted from **inactive** to **active** state
  - Adhesion to collagen
    - Triggers platelet activation
    - Tromboxane A_2 is synthesized from arachidonic acid and stimulates secretion
  - Aggregation of platelets to each other
    - prostacyclin (PGI_2) inhibits platelet aggregation
  - Secretion (discharge of granule contents)

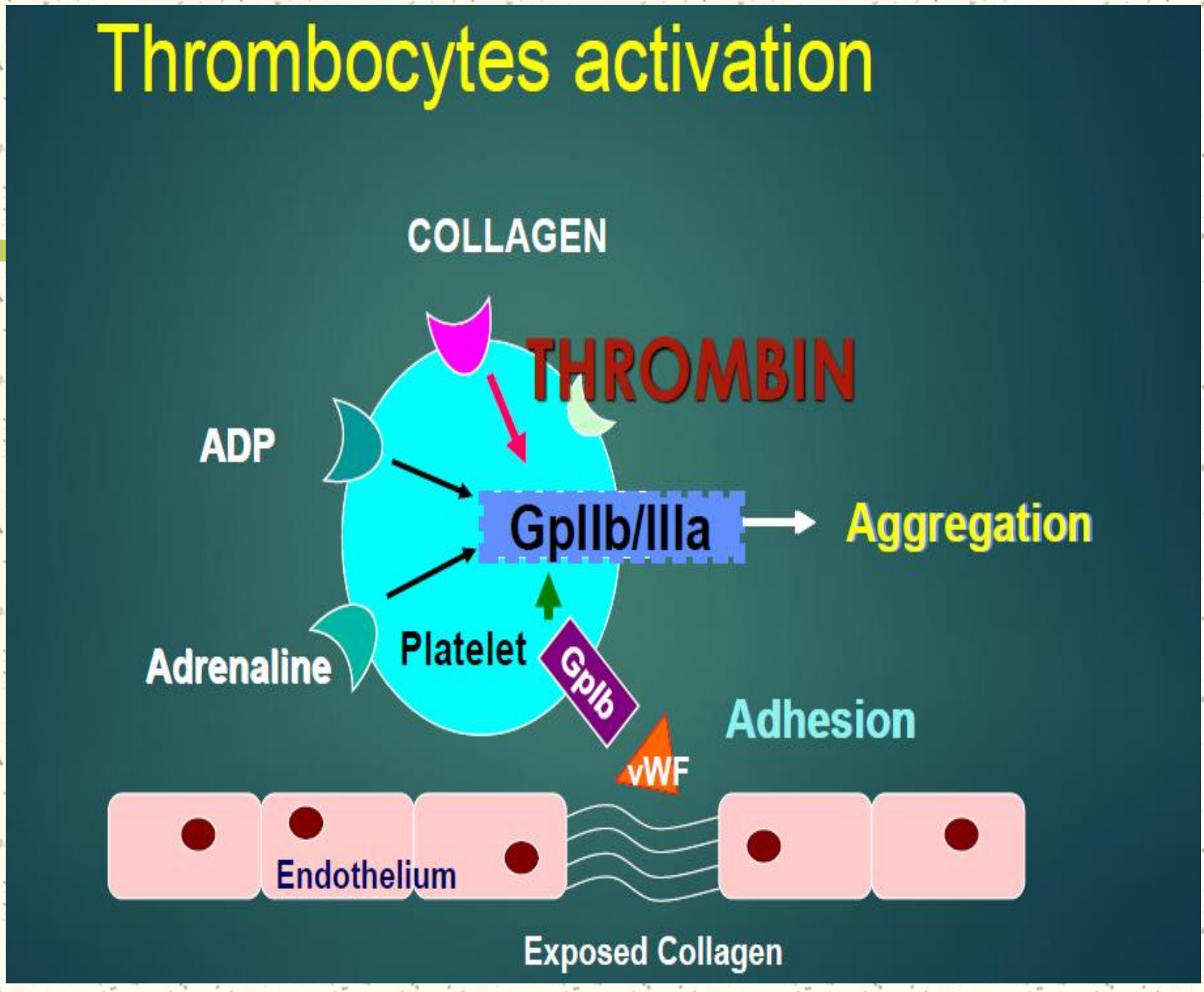


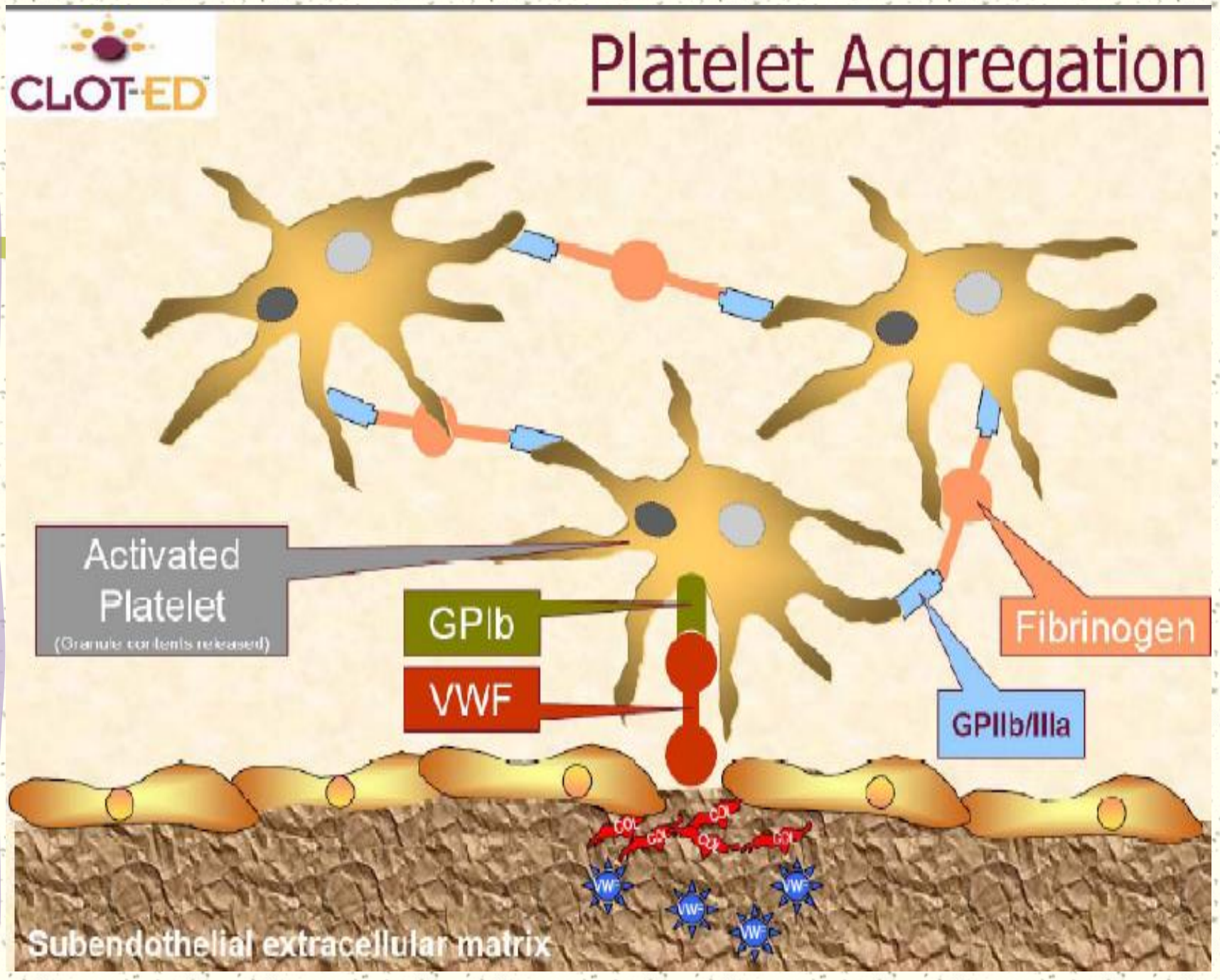

CLOT-ED
Platelet Adhesion
Platelet
GPIb
VWF
Endothelium
Injury site
collagen exposed
COL
VWF
Subendothelial extracellular matrix

Inactive

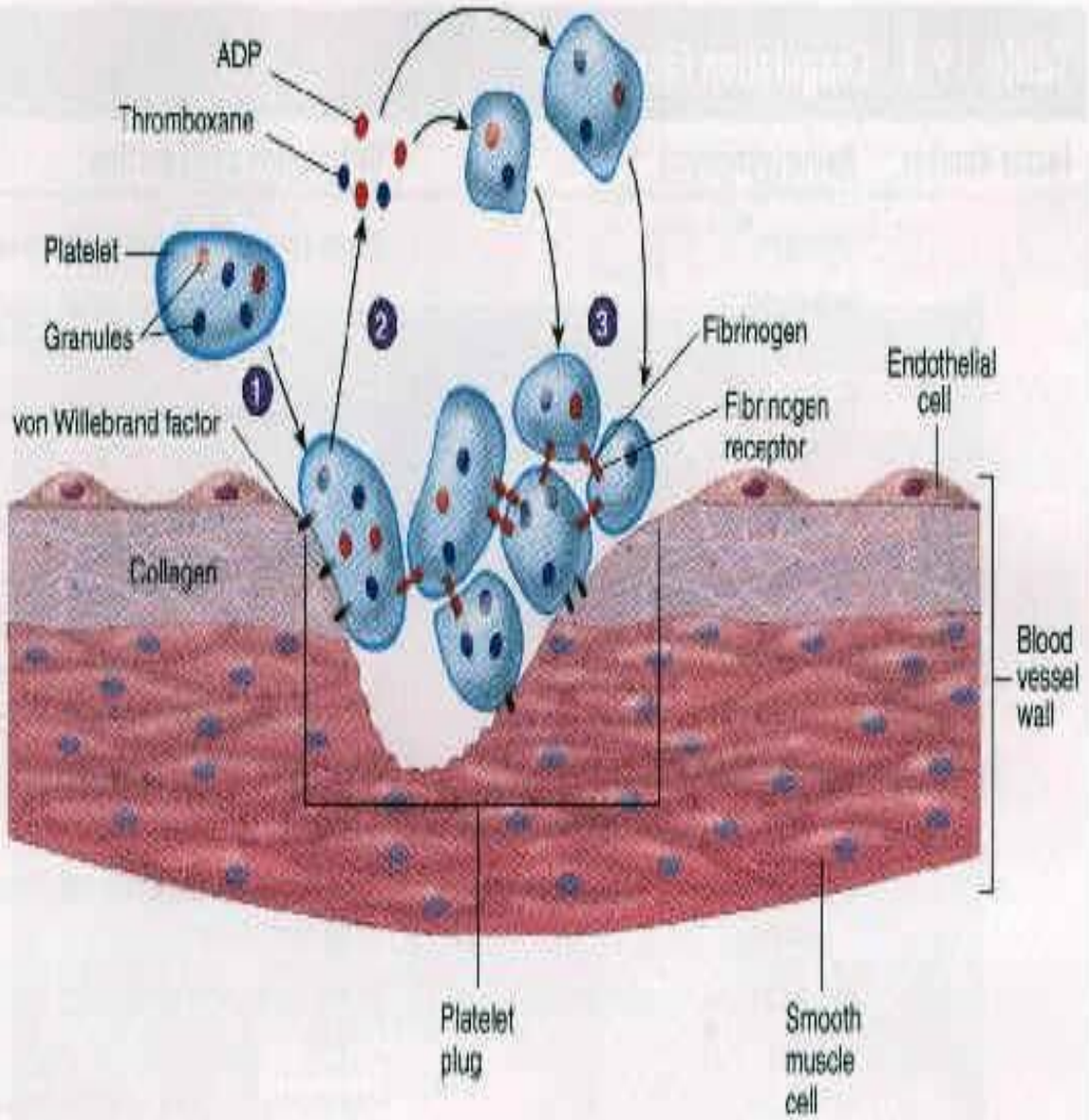
Active



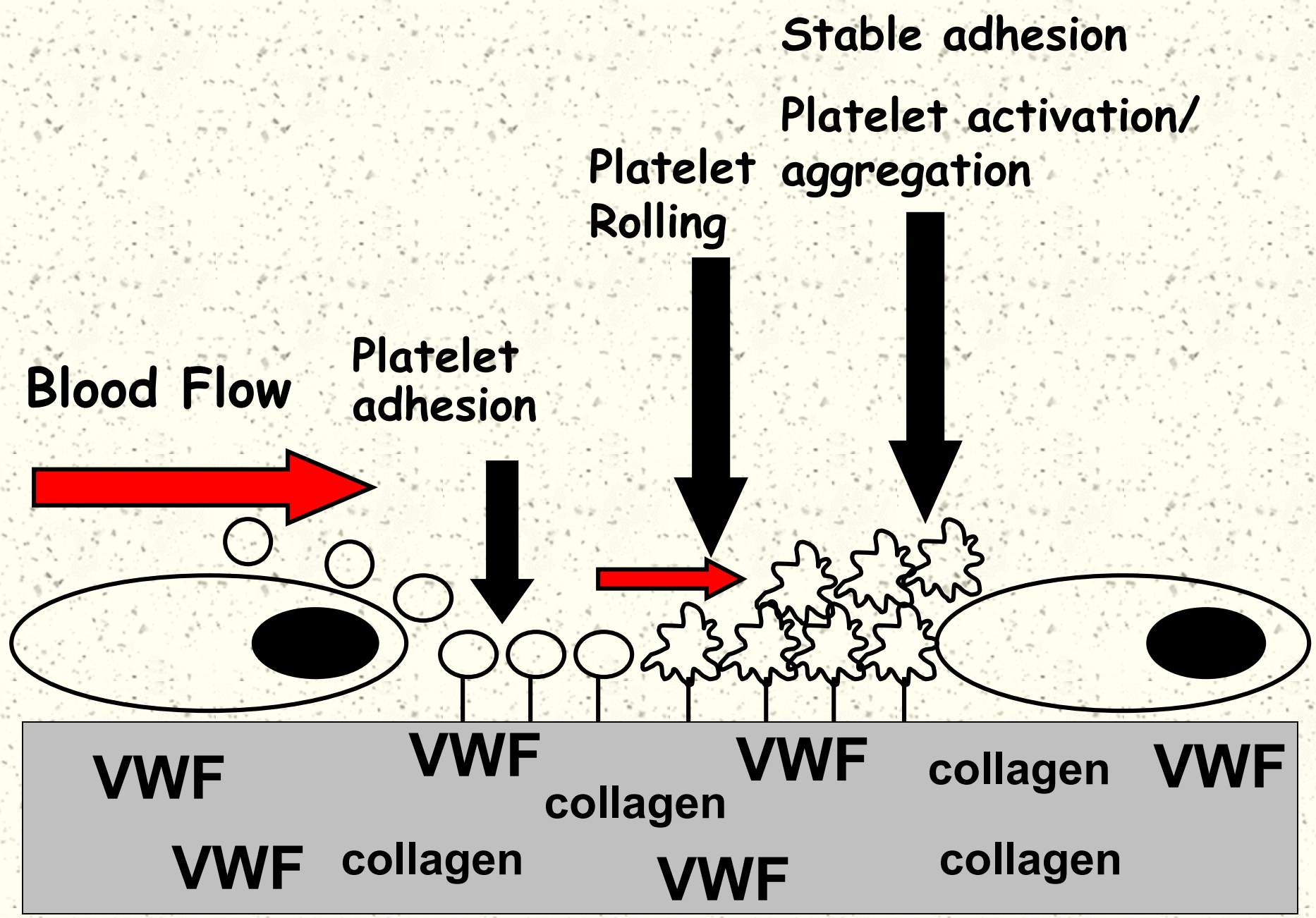

Thrombocytes activation
COLLAGEN
THROMBIN
ADP
GpIIb/IIIa
Aggregation
Adrenaline
Platelet
GpIb
Adhesion
vWF
Endothelium
Exposed Collagen

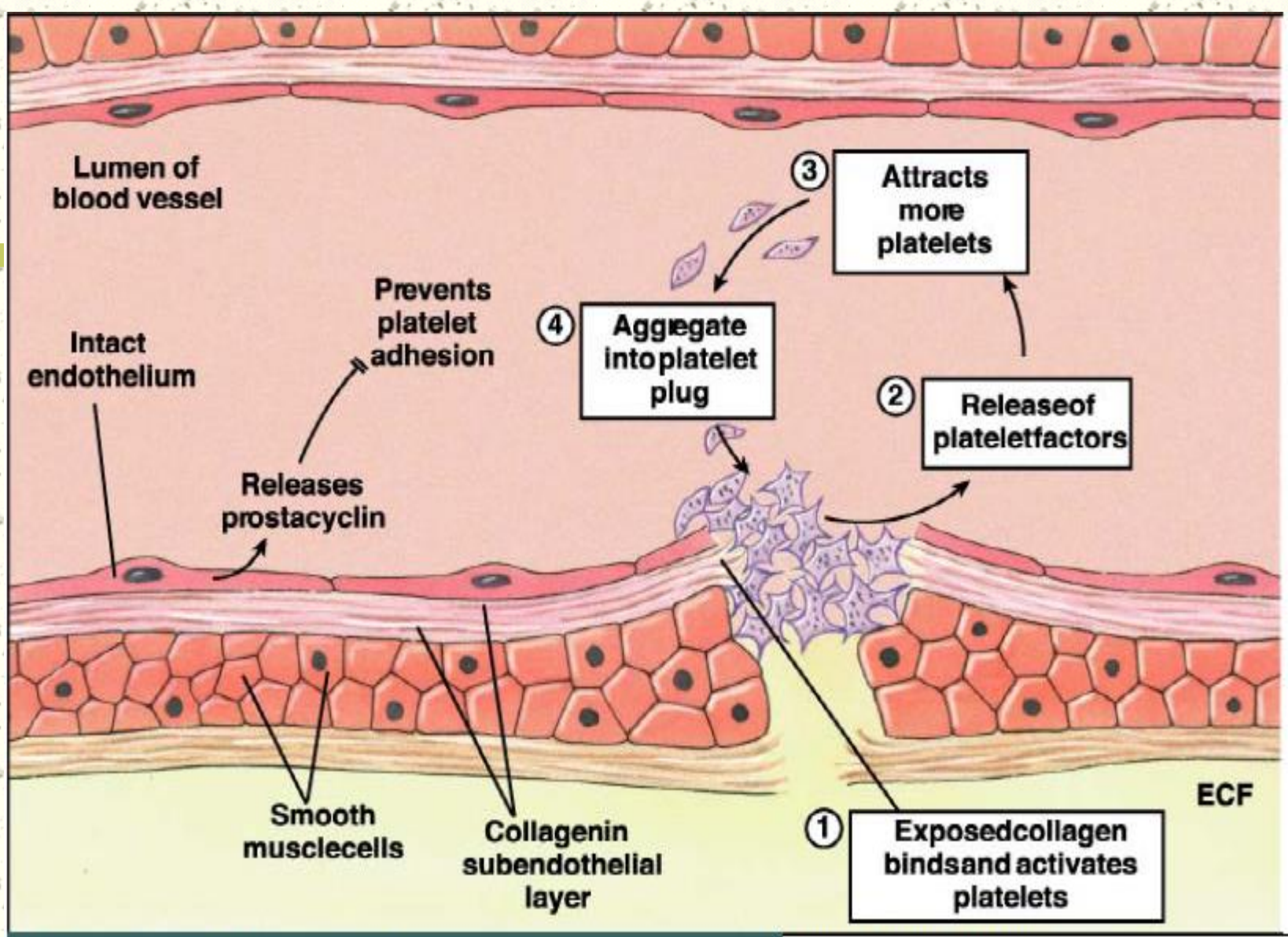

CLOT-ED
Platelet Aggregation
Activated Platelet
(Granule contents released)
GPIb
VWF
Fibrinogen
GPIIb/IIIa
Subendothelial extracellular matrix

1. Platelet adhesion occurs when von Willebrand factor connects collagen and platelets.
2. The platelet release reaction is the release of ADP, thromboxanes, and other chemicals that activate other platelets.
3. Platelet aggregation occurs when fibrinogen receptors on activated platelets bind to fibrinogen, connecting the platelets to one another. A platelet plug is formed by the accumulating mass of platelets.



Platelet Plug Formation

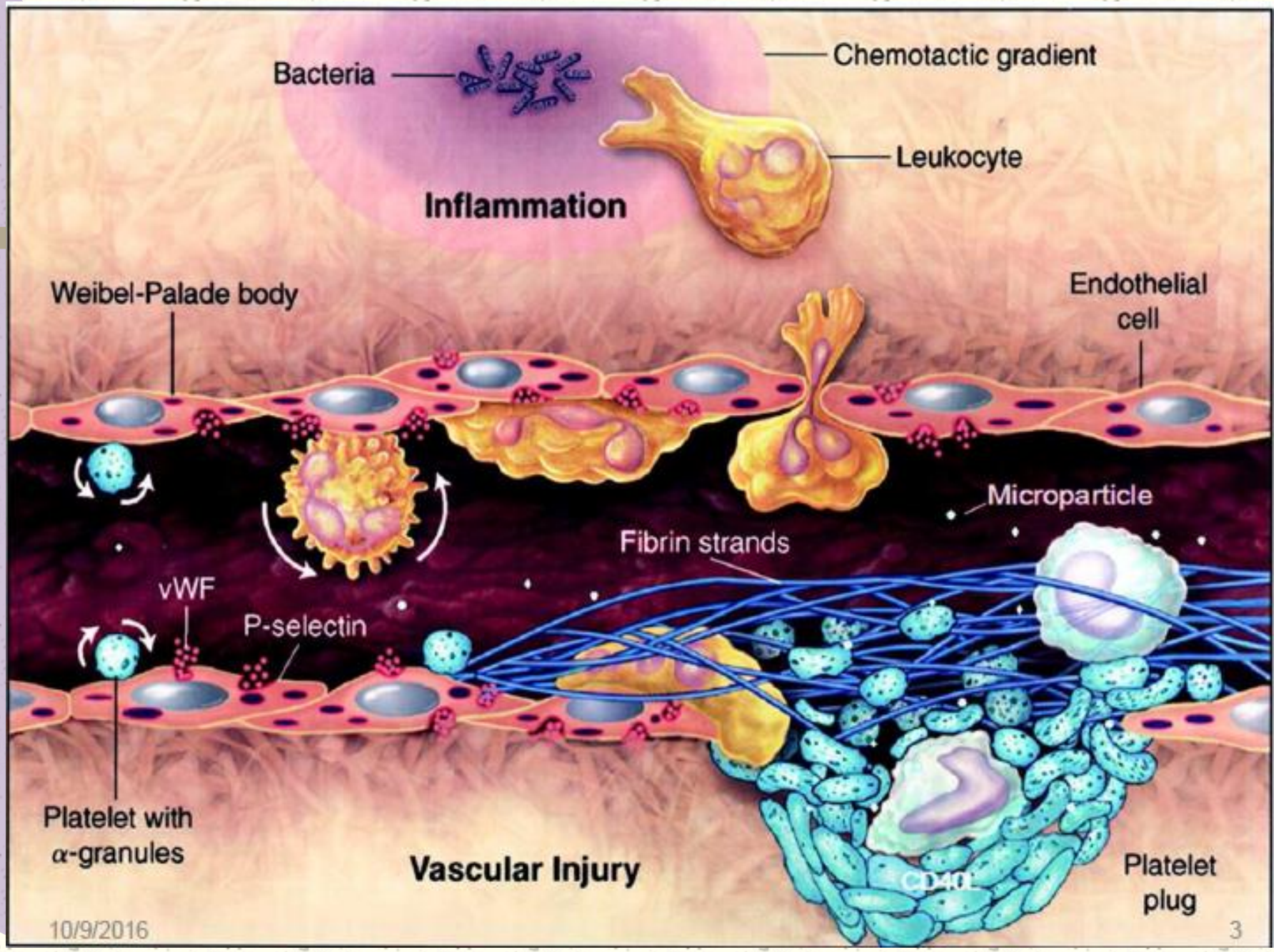

Stable adhesion
Platelet activation/ aggregation
Platelet Rolling
Blood Flow
Platelet adhesion
VWF
VWF
VWF
collagen
VWF
collagen
VWF
collagen
VWF
collagen

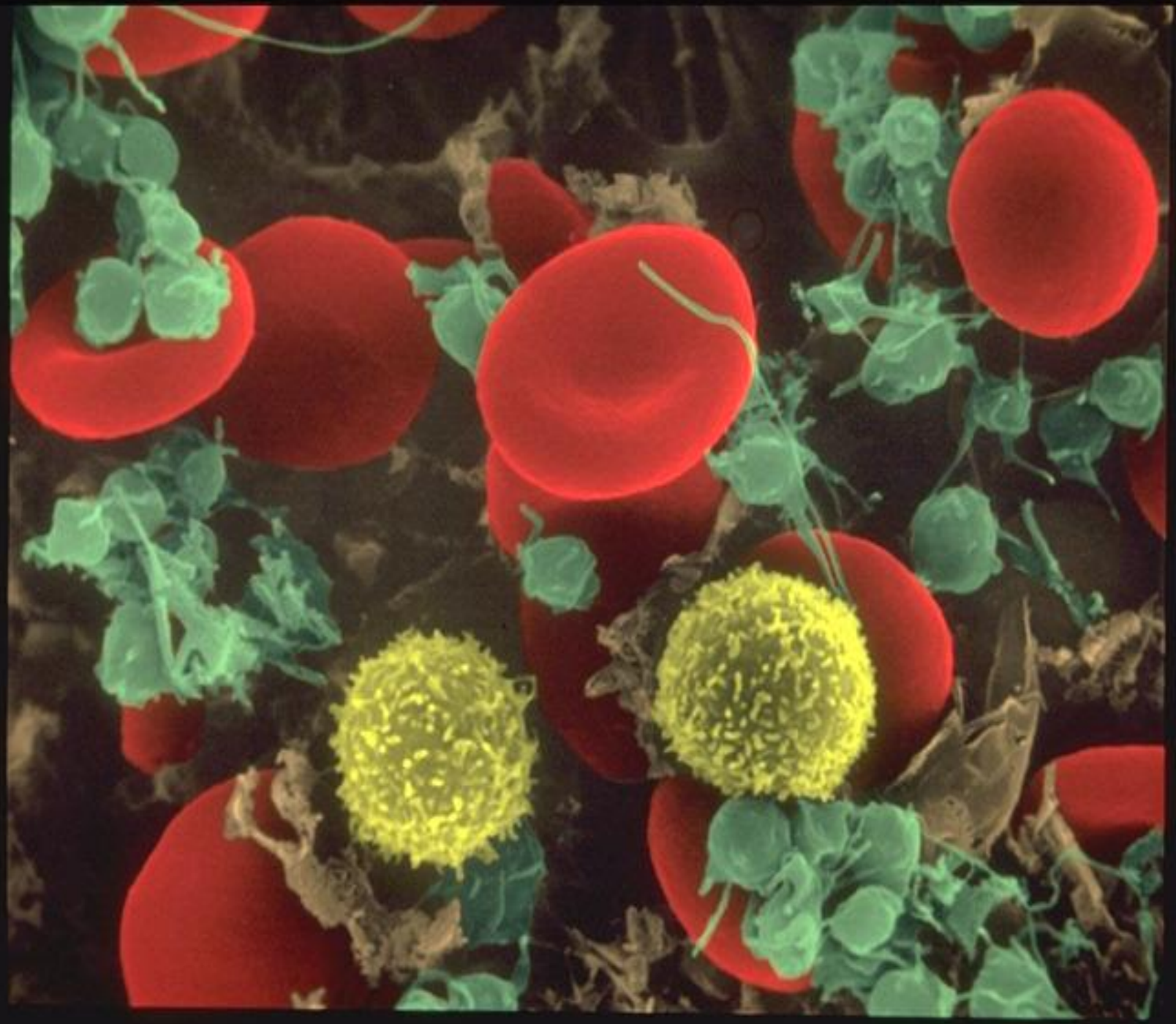


Overview of Hemostasis: Clot Formation & Vessel Repair

Platelet Plug Formation

- Platelets do not stick to each other or to blood vessels

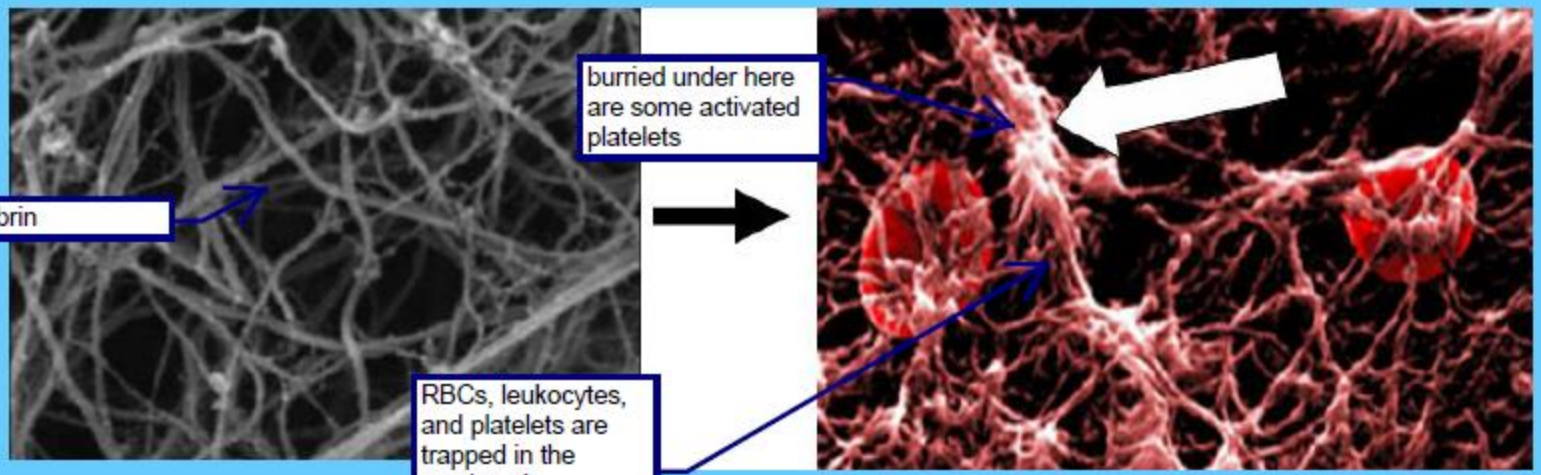

Bacteria
Chemotactic gradient
Leukocyte
Inflammation
Weibel-Palade body
Endothelial cell
Microparticle
Fibrin strands
vWF
P-selectin
Platelet with α-granules
Vascular Injury
CD40
Platelet plug



Secondary Hemostasis

point of secondary hemostasis is to consolidate platelet plug in a fibrin meshwork

Coagulation proteins act on platelet surfaces to form fibrin, which stabilizes the platelet plug



3. Blood coagulation

- Begins ~ 15-20 s to 1 min after vascular damage
- Initiated by:
  - Release of active factors from injured vessel wall
  - Activated platelets
  - Blood proteins adhering to damaged vessel wall
- If vessel opening is not too large, in 3-6 min the bleeding is stopped.
- In 20 min – clot retraction.

3-Blood Coagulation

- Is the process where by on vessel injury, Plasma protein, Tissue factors and Calcium interact on the surface of the platelets to form a *Fibrin clot*.
- Platelets provide a surface for the coagulation reaction, and interact with fibrin to form a stable platelet fibrin clot.

Importance of Calcium ions

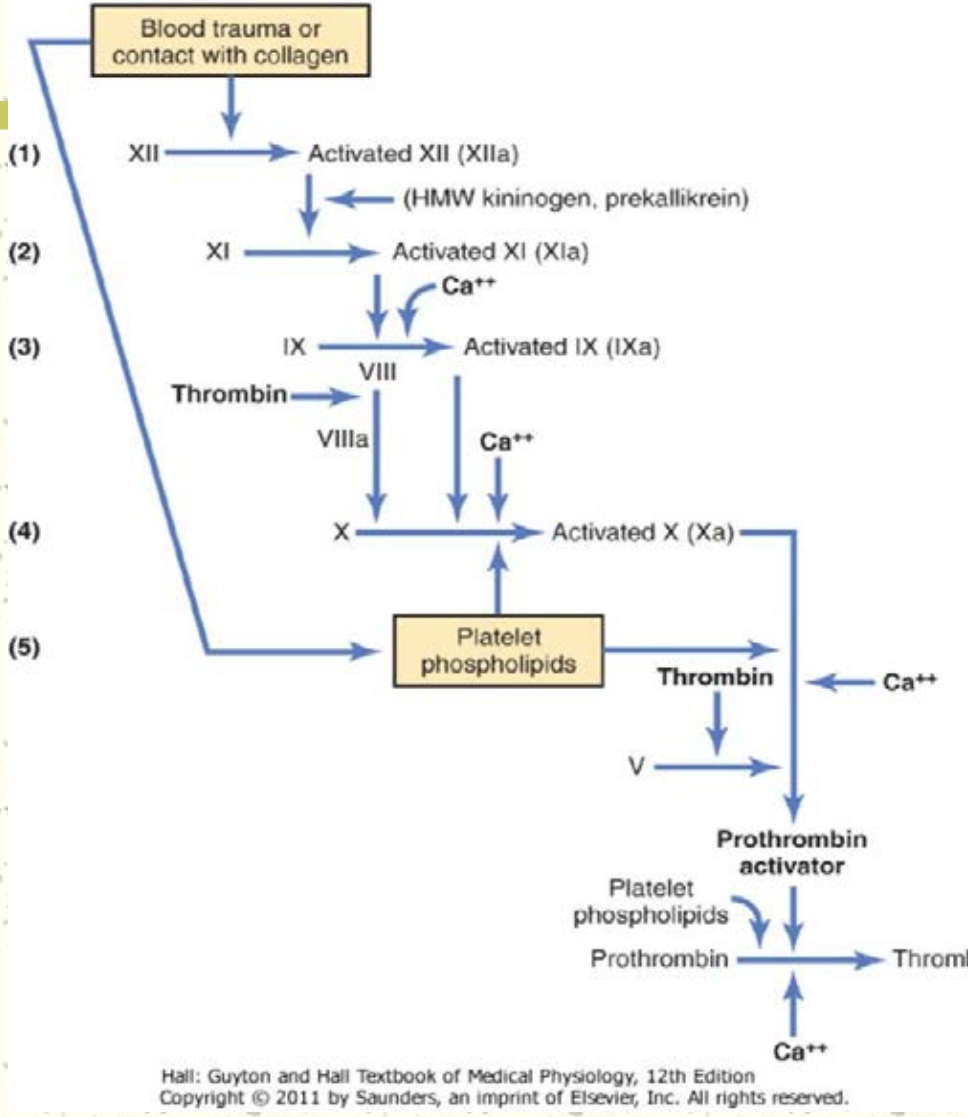
- Except for the first two steps in the intrinsic pathway, calcium ions are required for promotion or acceleration of all the blood-clotting reactions.
- When blood is removed from a person, it can be prevented from clotting by reducing the calcium ion concentration.
- The extrinsic pathway can be explosive. The intrinsic pathway is much slower to proceed, usually requiring 1 to 6 minutes to cause clotting.

Intrinsic pathway

- Starts with trauma to the blood or contact of blood with collagen
- More steps in cascade and thus slower than extrinsic pathway.
- All coagulation factors are in the blood.

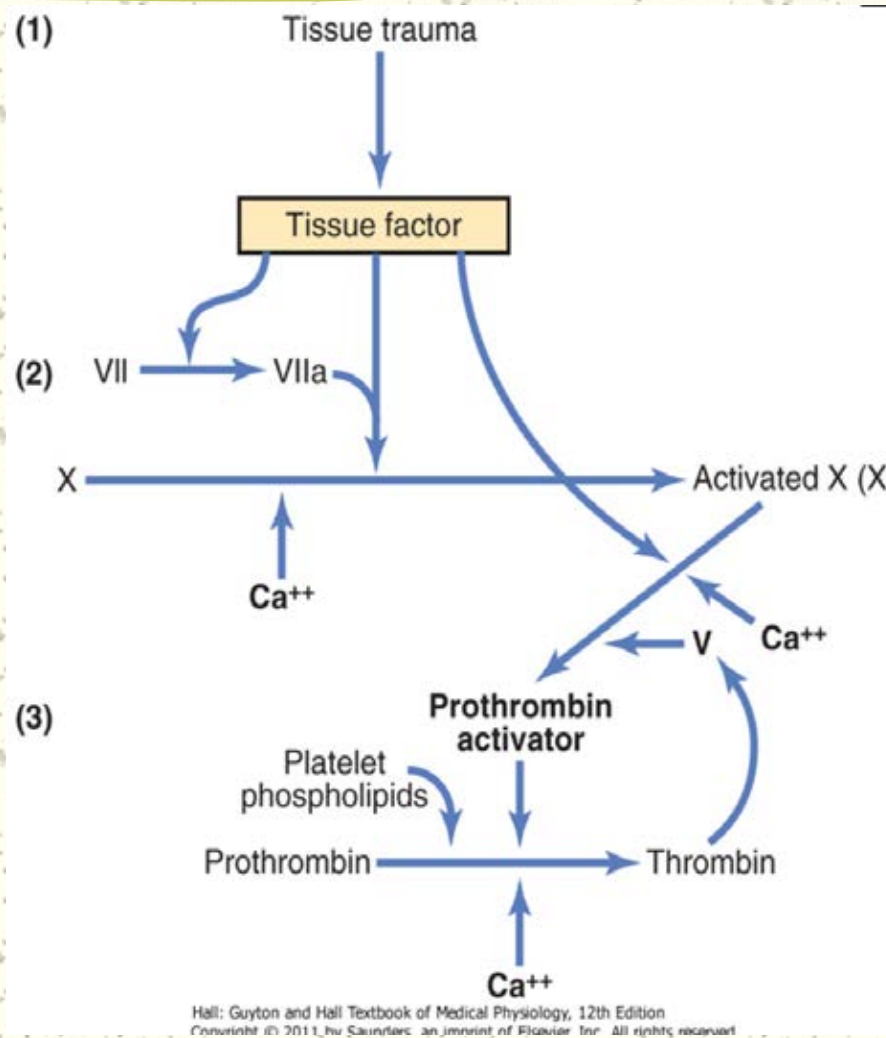
Intrinsic pathway

- Factor VIII – *antihemophyilic factor*
- Ca^{2+} necessary for all, but first two steps
- removal of calcium (citrate, oxalate) – prevention of clotting



Extrinsic Pathway

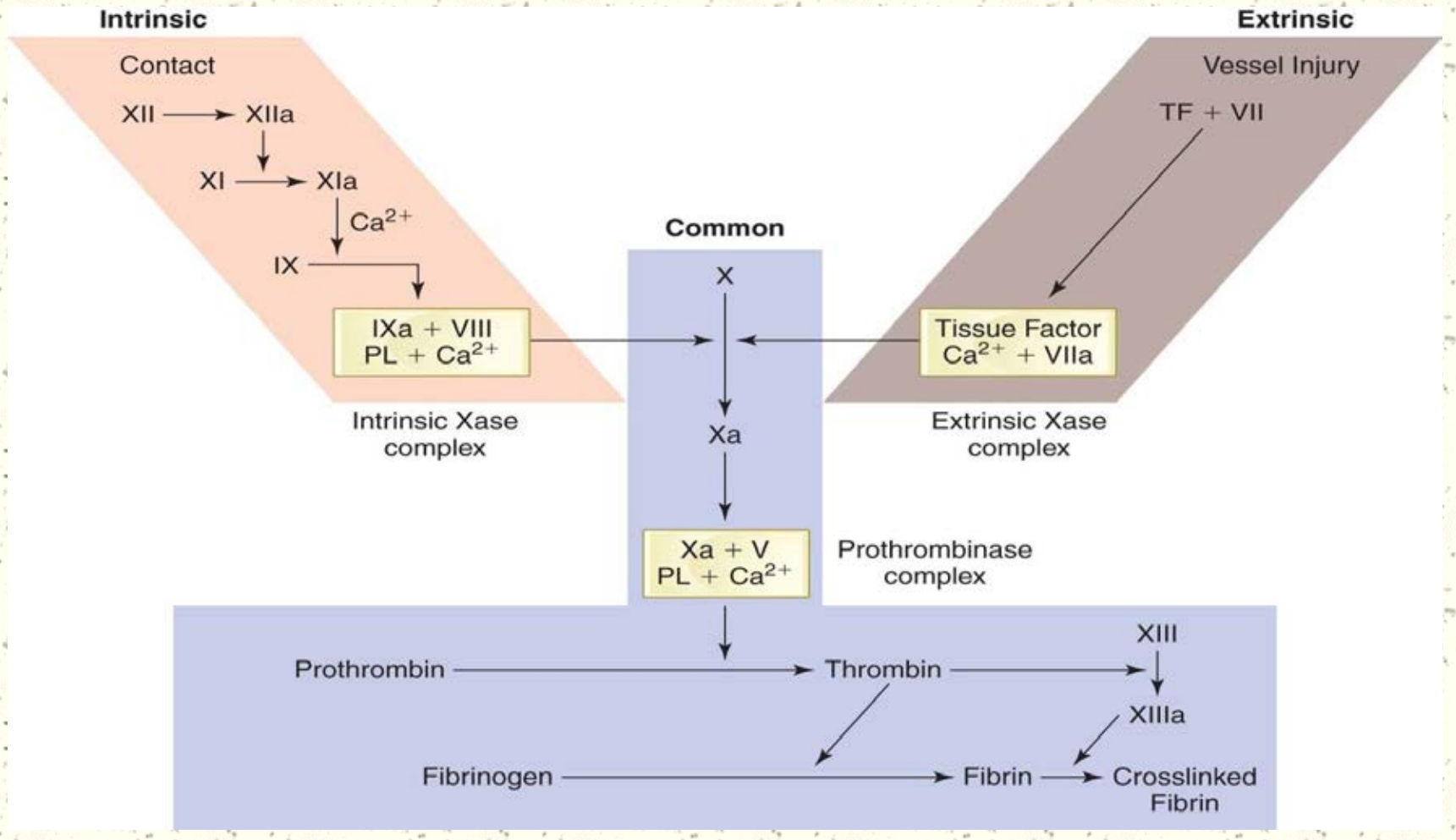
- Initiates when injured vascular wall or extravascular tissues come in contact with blood.
- Injured tissues release *tissue factor (tissue thromboplastin)* - lipoprotein (proteolytic) and phospholipid component

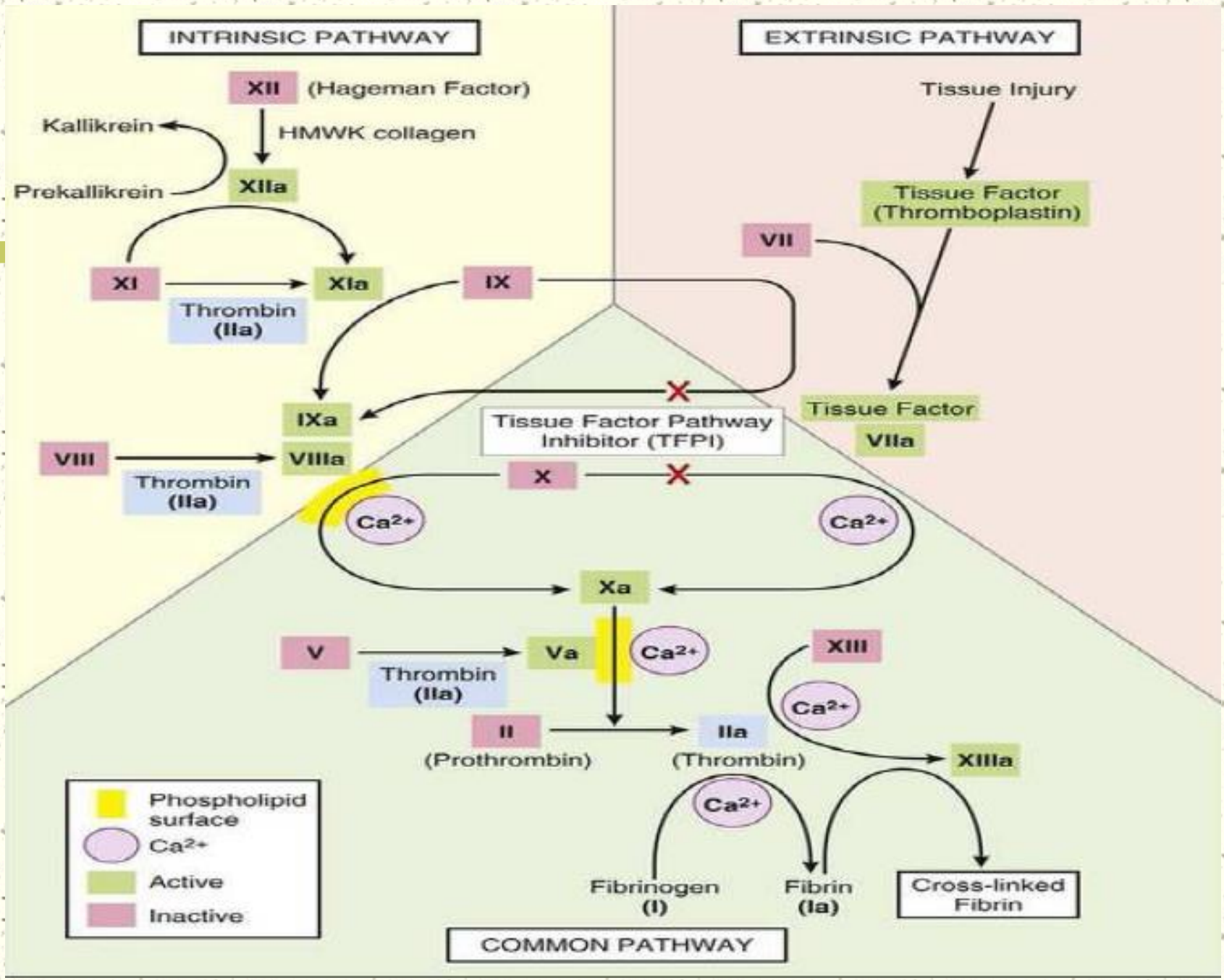


Interaction between extrinsic and intrinsic pathways

- Damage of blood vessel activates both pathways!
- They converge at the level of factor X.
- Extrinsic pathway – explosive (15 s).
- Intrinsic patway – slower (1-6 min).

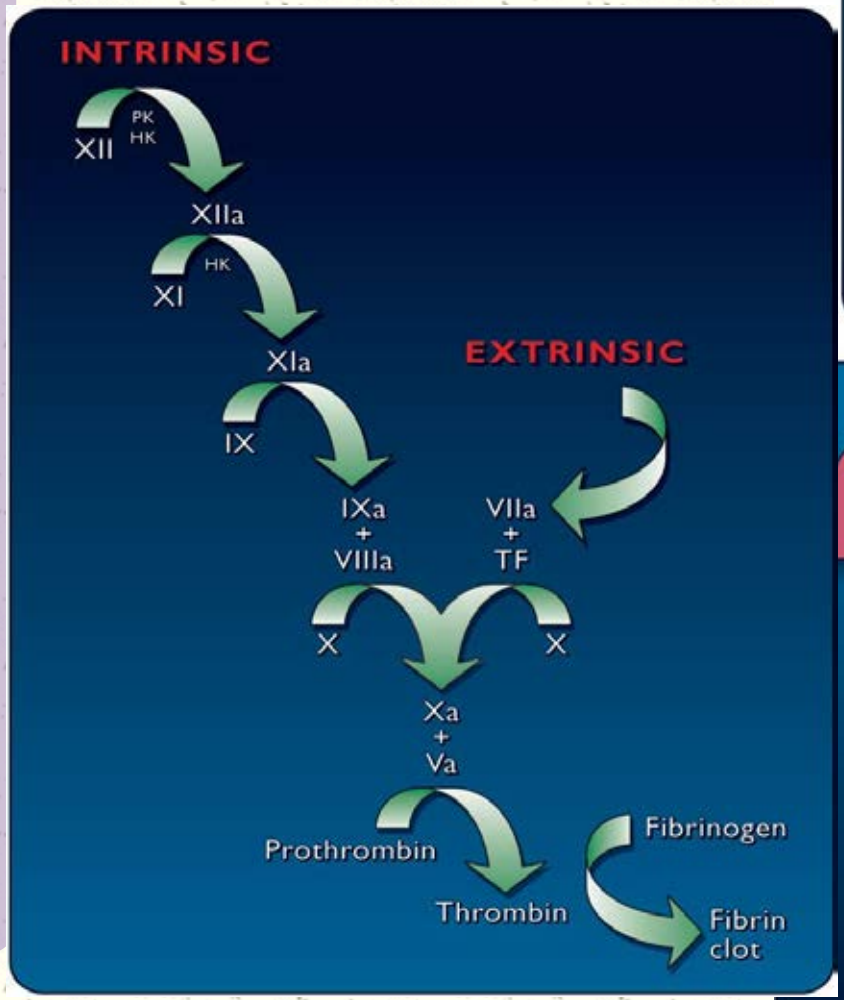
Coagulation Cascade

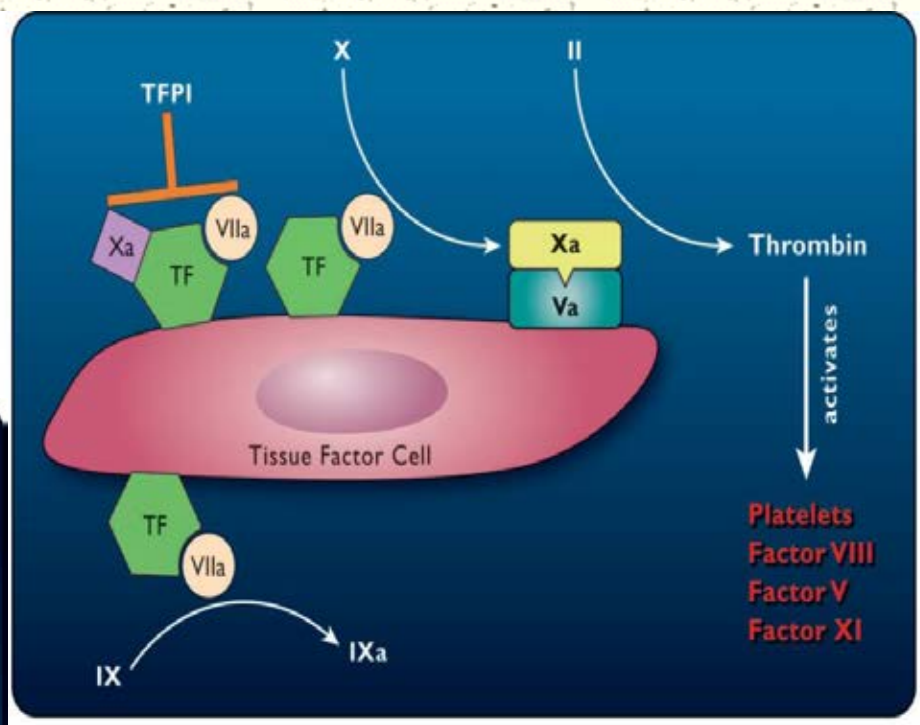


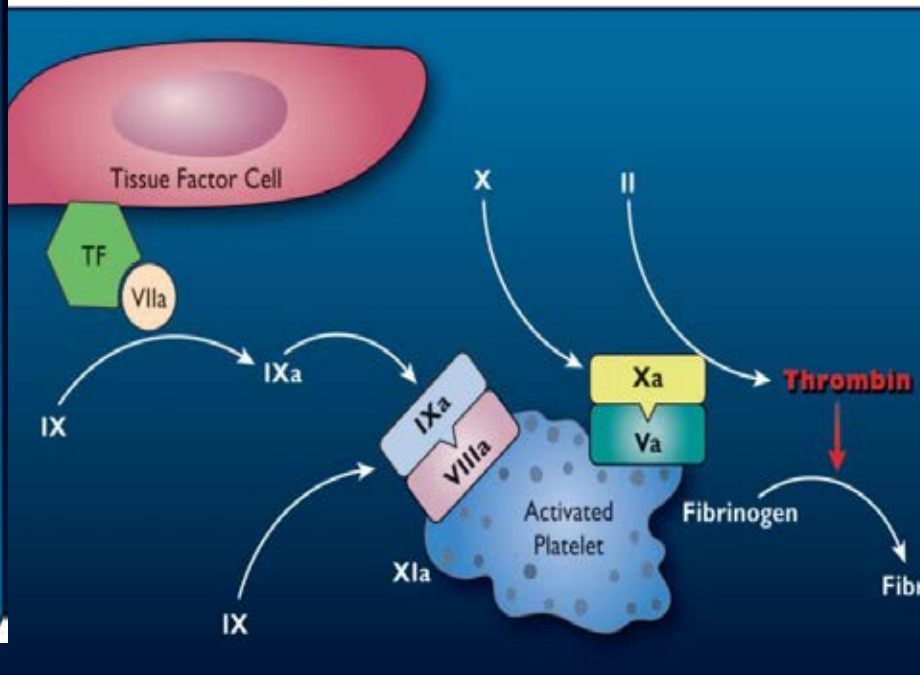

INTRINSIC PATHWAY
EXTRINSIC PATHWAY
XII (Hageman Factor)
Kallikrein
HMWK collagen
Prekallikrein
XIIa
Tissue Injury
Tissue Factor (Thromboplastin)
VII
XI
XIa
Thrombin (IIa)
IX
IXa
Tissue Factor Pathway Inhibitor (TFPI)
Tissue Factor
VIIa
VIII
VIIIa
Thrombin (IIa)
X
Ca2+
Ca2+
Xa
V
Va
Ca2+
Thrombin (IIa)
XIII
Ca2+
II
(Prothrombin)
IIa
(Thrombin)
XIIIa
Phospholipid surface
Ca2+
Active
Inactive
Ca2+
Fibrinogen (I)
Fibrin (Ia)
Cross-linked Fibrin
COMMON PATHWAY

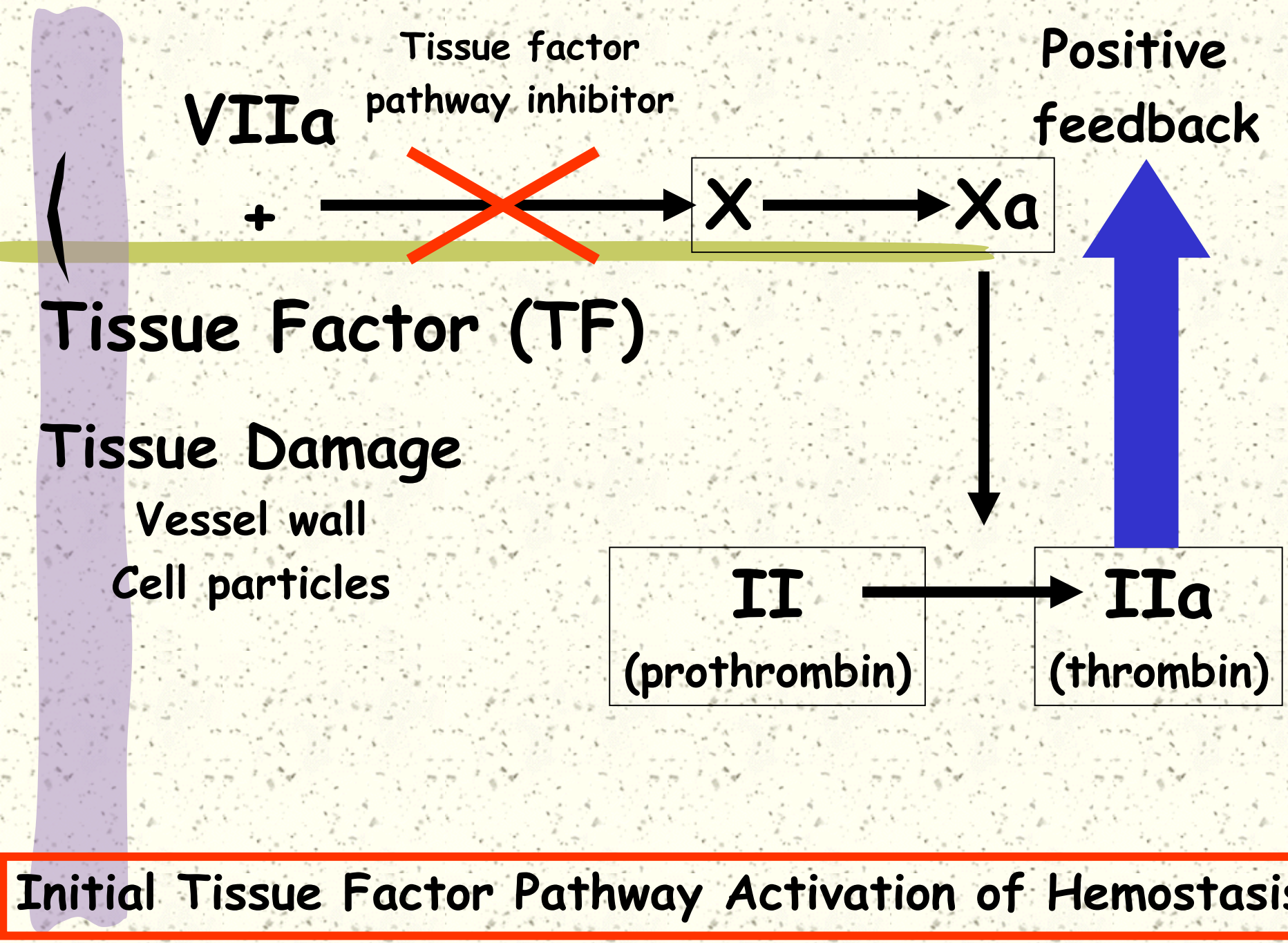
Breakdown of Factor Location

Intrinsic Pathway	Extrinsic Pathway	Common Pathway
Prekallikrein= PK	VII	X
HK	Tissue factor= TF, III	V
XII		II
XI		I
IX		
VIII		

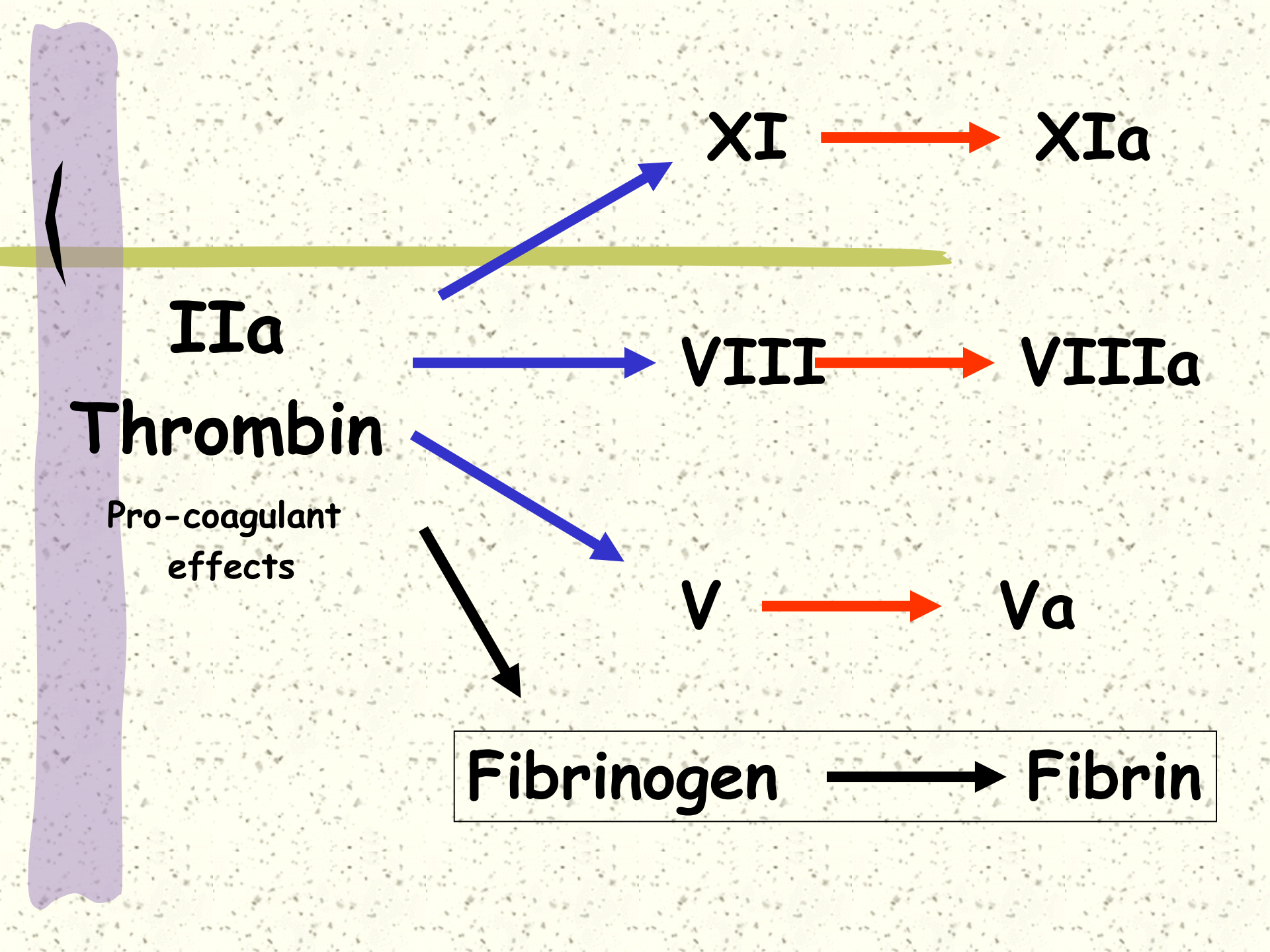

INTRINSIC
XII
PK
HK
XIIa
XI
HK
XIa
IX
IXa
+
VIIIa
EXTRINSIC
VIIa
+
TF
X
X
Xa
+
Va
Prothrombin
Thrombin
Fibrinogen
Fibrin
clot

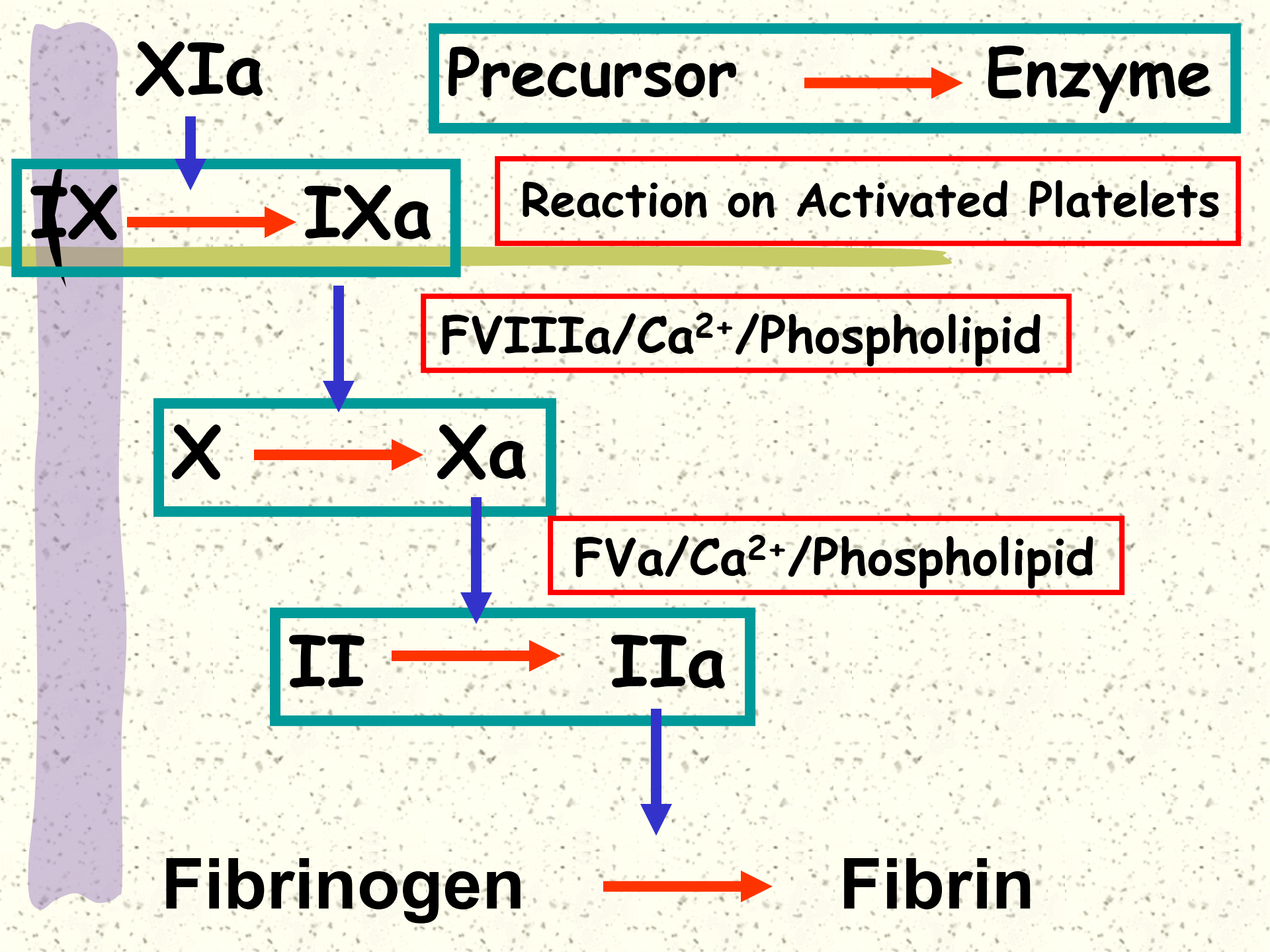

TFPI
X
II
Xa
TF
VIIa
TF
VIIa
Xa
Va
Thrombin
activates
Tissue Factor Cell
TF
VIIa
IX
IXa
Platelets
Factor VIII
Factor V
Factor XI

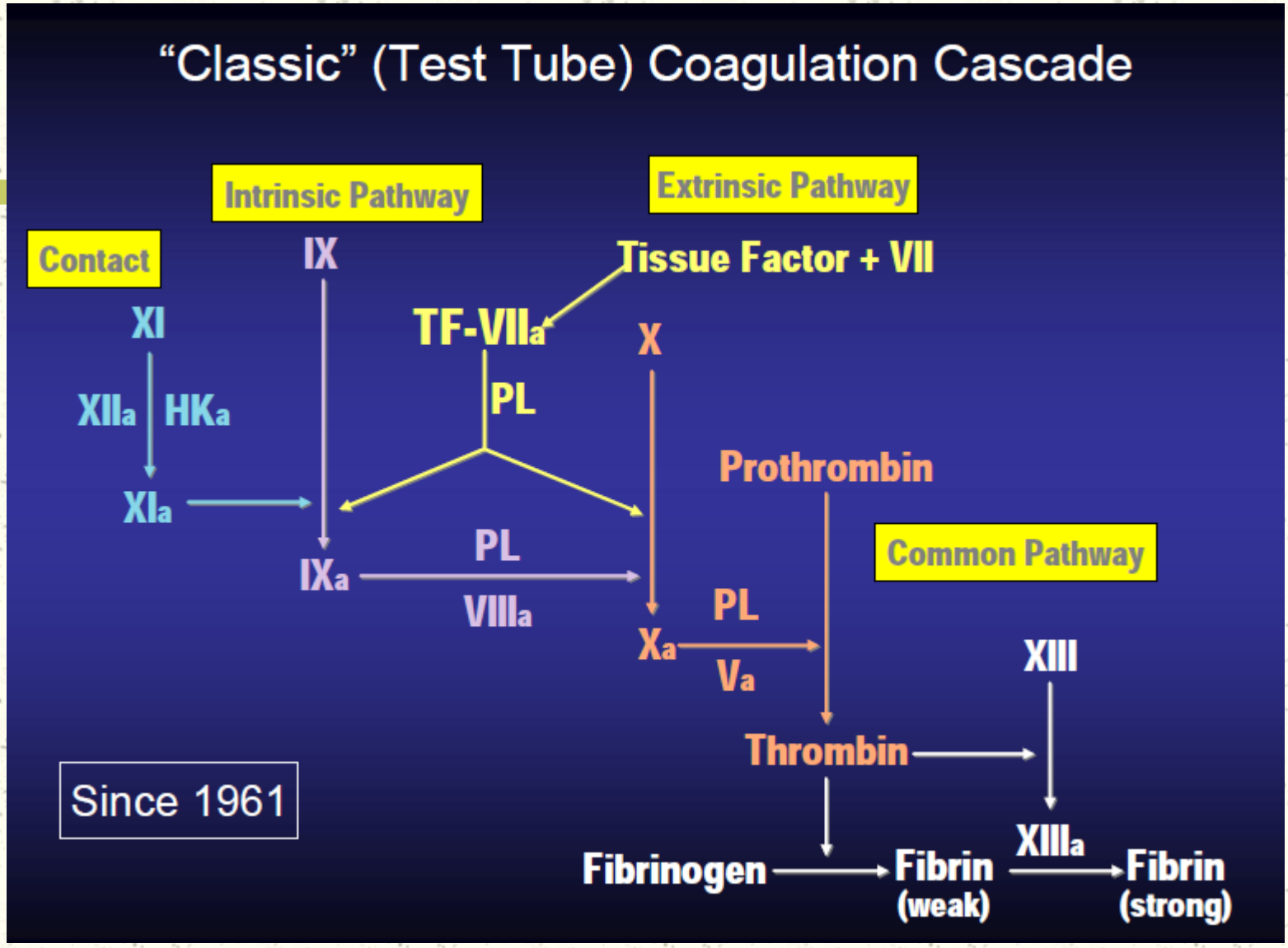

Tissue Factor Cell
TF
VIIa
X
II
IX
IXa
IXa
VIIIa
Xa
Va
Thrombin
Activated
Platelet
Fibrinogen
XIa
IX

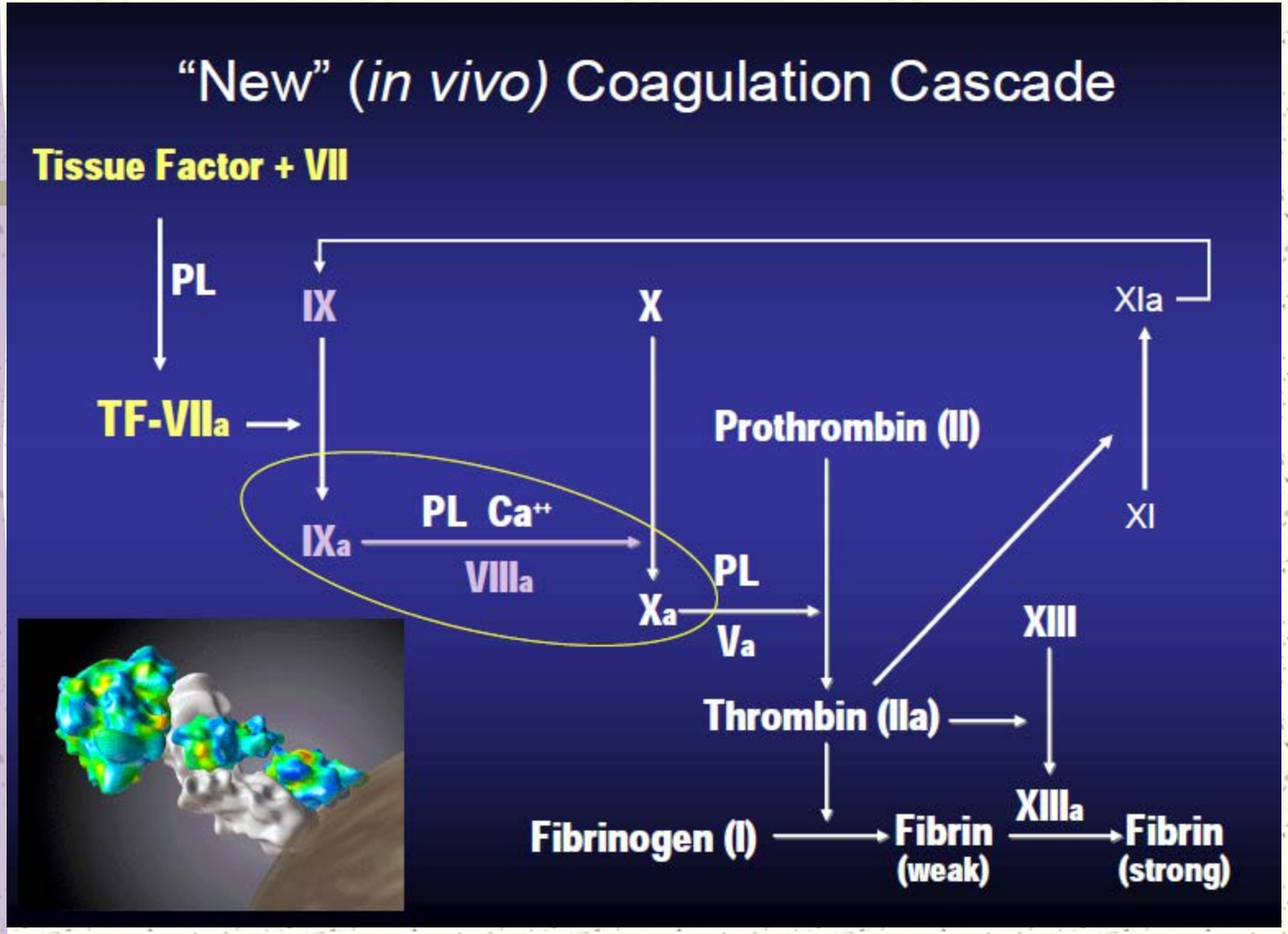


Initial Tissue Factor Pathway Activation of Hemostasis


XI
XIa
IIa
Thrombin
Pro-coagulant
effects
VIII
VIIIa
V
Va
Fibrinogen
Fibrin


XIa
Precursor
Enzyme
IX
IXa
Reaction on Activated Platelets
FVIIIa/Ca2+/Phospholipid
X
Xa
FVa/Ca2+/Phospholipid
II
IIa
Fibrinogen
Fibrin


"Classic" (Test Tube) Coagulation Cascade
Intrinsic Pathway
Extrinsic Pathway
Contact
IX
Tissue Factor + VII
XI
TF-VIIa
X
XIIa
HKa
PL
Prothrombin
XIa
PL
Common Pathway
IXa
VIIIa
PL
Xa
Va
XIII
Thrombin
Since 1961
XIIIa
Fibrinogen
Fibrin
(weak)
Fibrin
(strong)


"New" (*in vivo*) Coagulation Cascade
Tissue Factor + VII
PL
IX
X
XIa
TF-VIIa
Prothrombin (II)
PL Ca++
IXa
VIIIa
XI
PL
Xa
Va
XIII
Thrombin (IIa)
XIIIa
Fibrinogen (I)
Fibrin (weak)
Fibrin (strong)

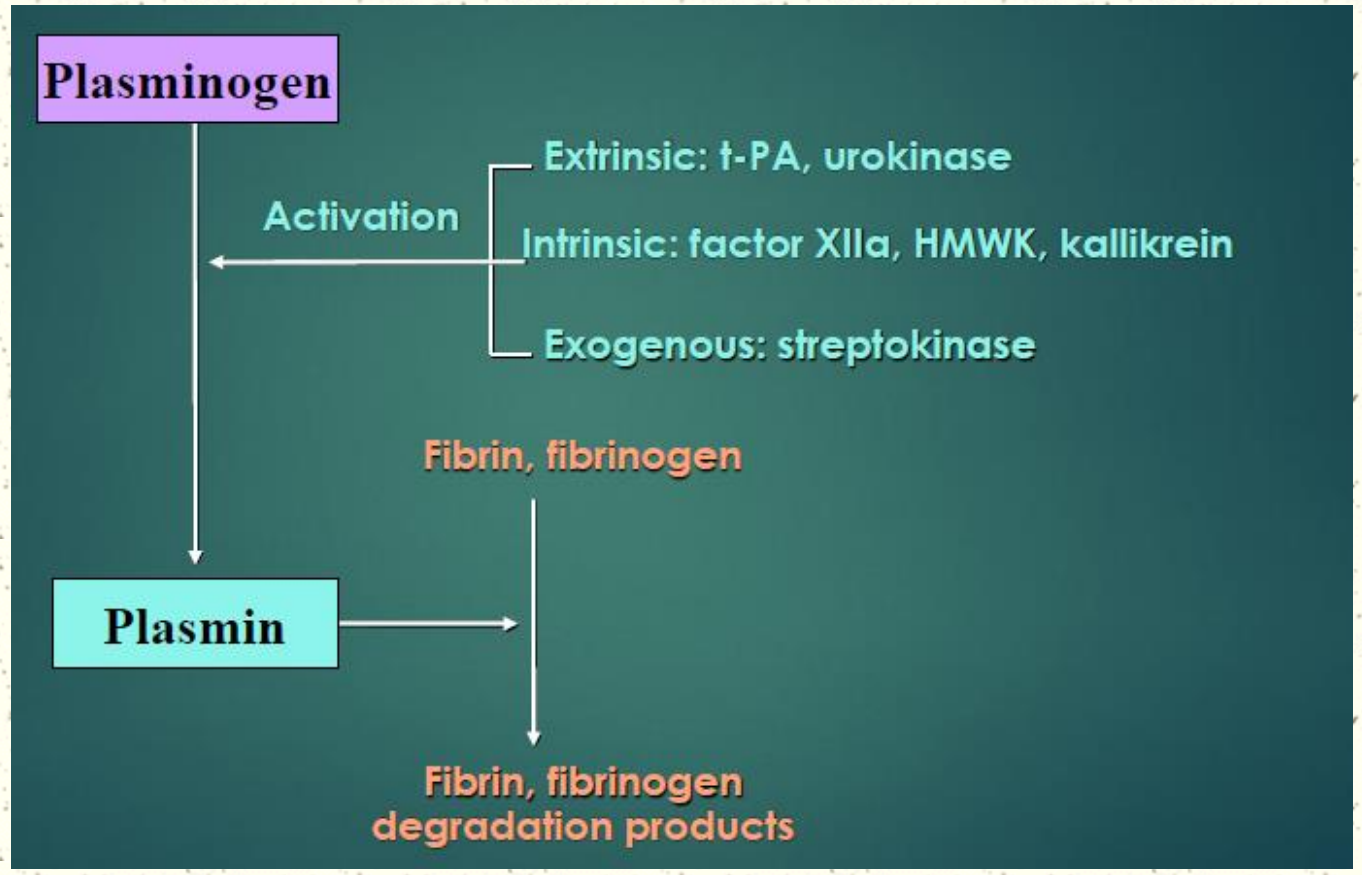
Clot retraction - Serum

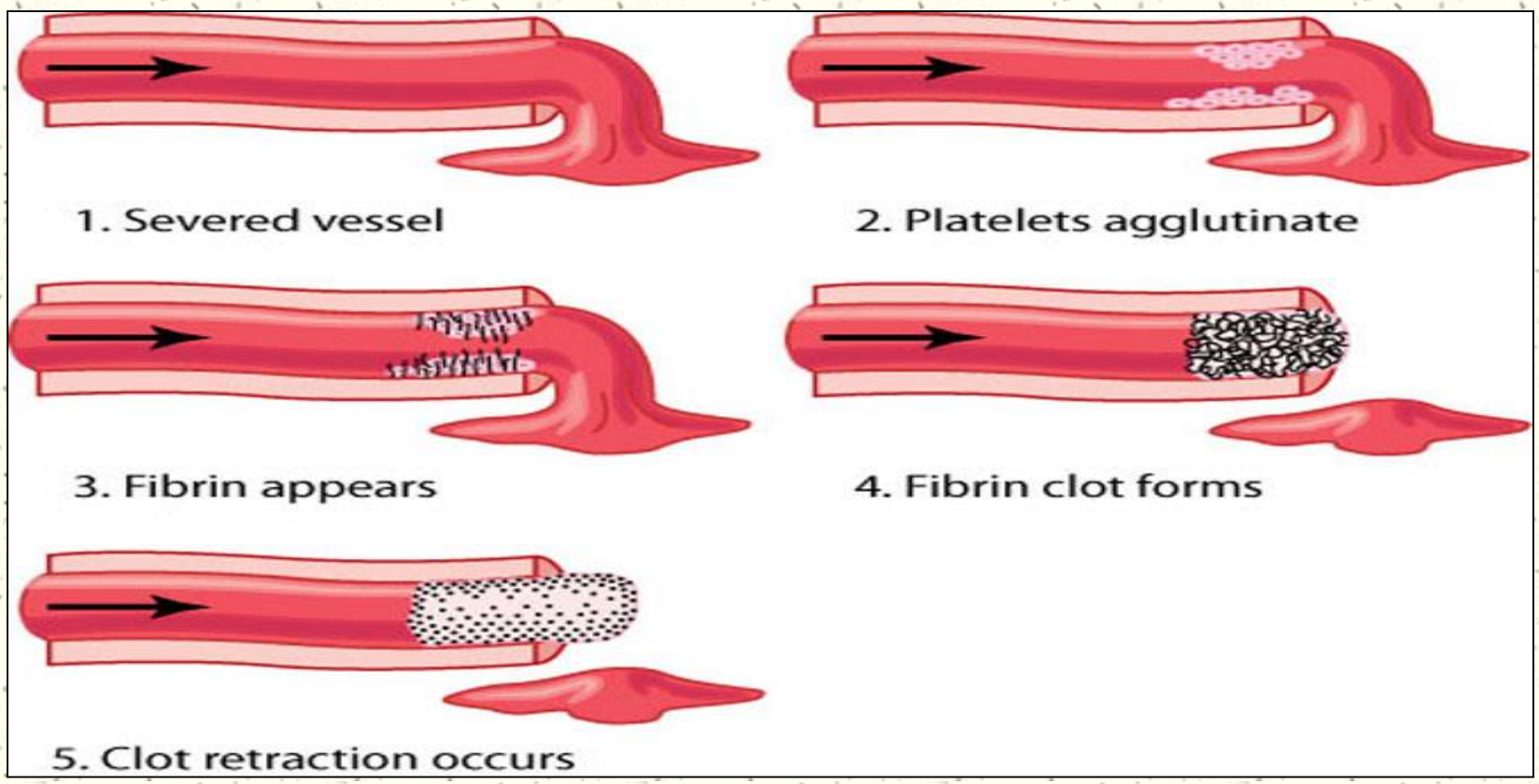
- 60 min after clot formation all the fluid is expressed out (serum)
- Platelets are essential for retraction and pulling together the edges of broken vessel.

FIBRINOLYTIC SYSTEM

- Definition: temporary fibrin clot systematically and gradually dissolved as the vessel heals
- Key components
  - Plasminogen (inactive form)
  - Plasminogen activators
  - Plasmin
  - Fibrin
  - Fibrin Degradation Products (FDP)
  - Inhibitors of plasminogen activators and plasmin

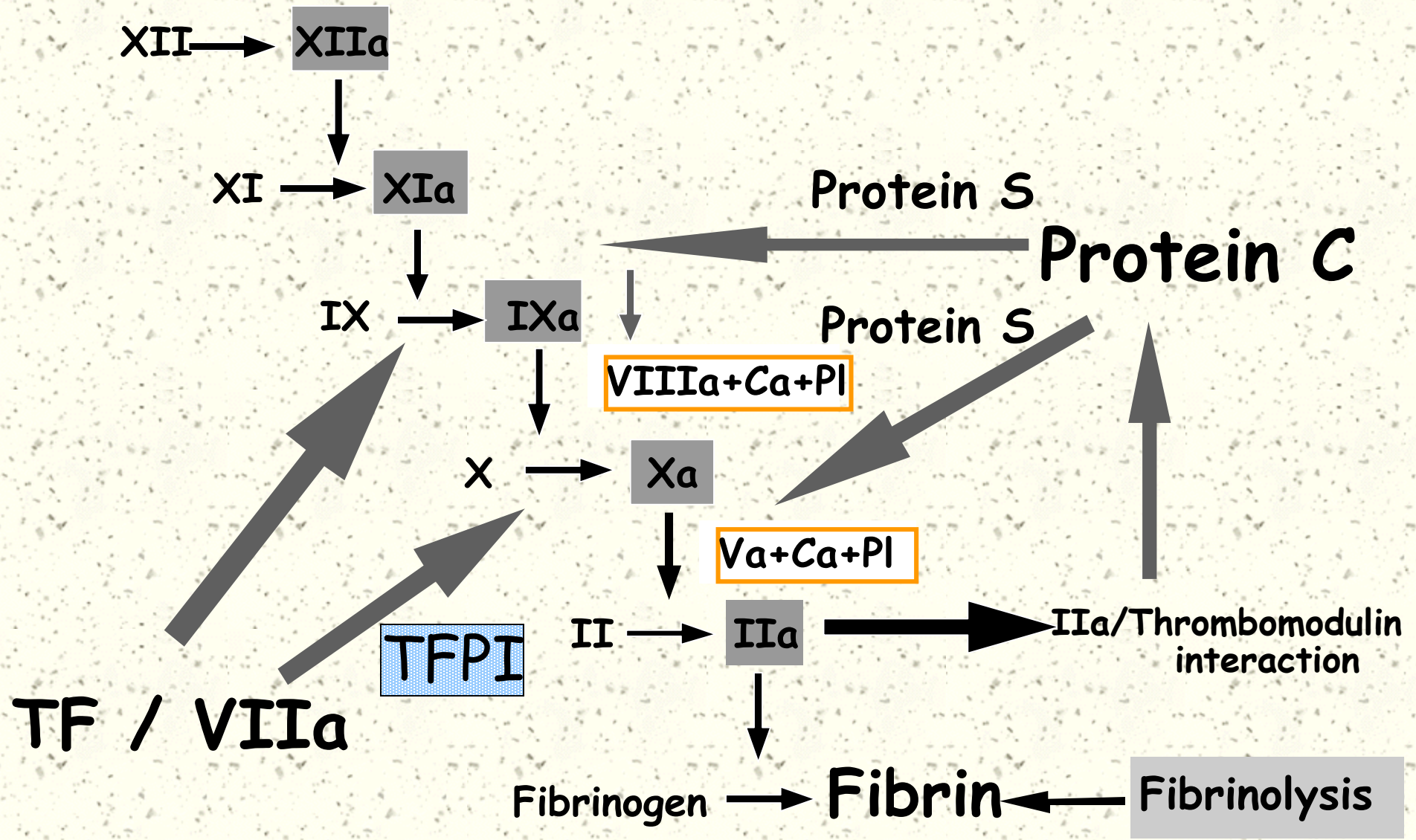
Fibriolysis

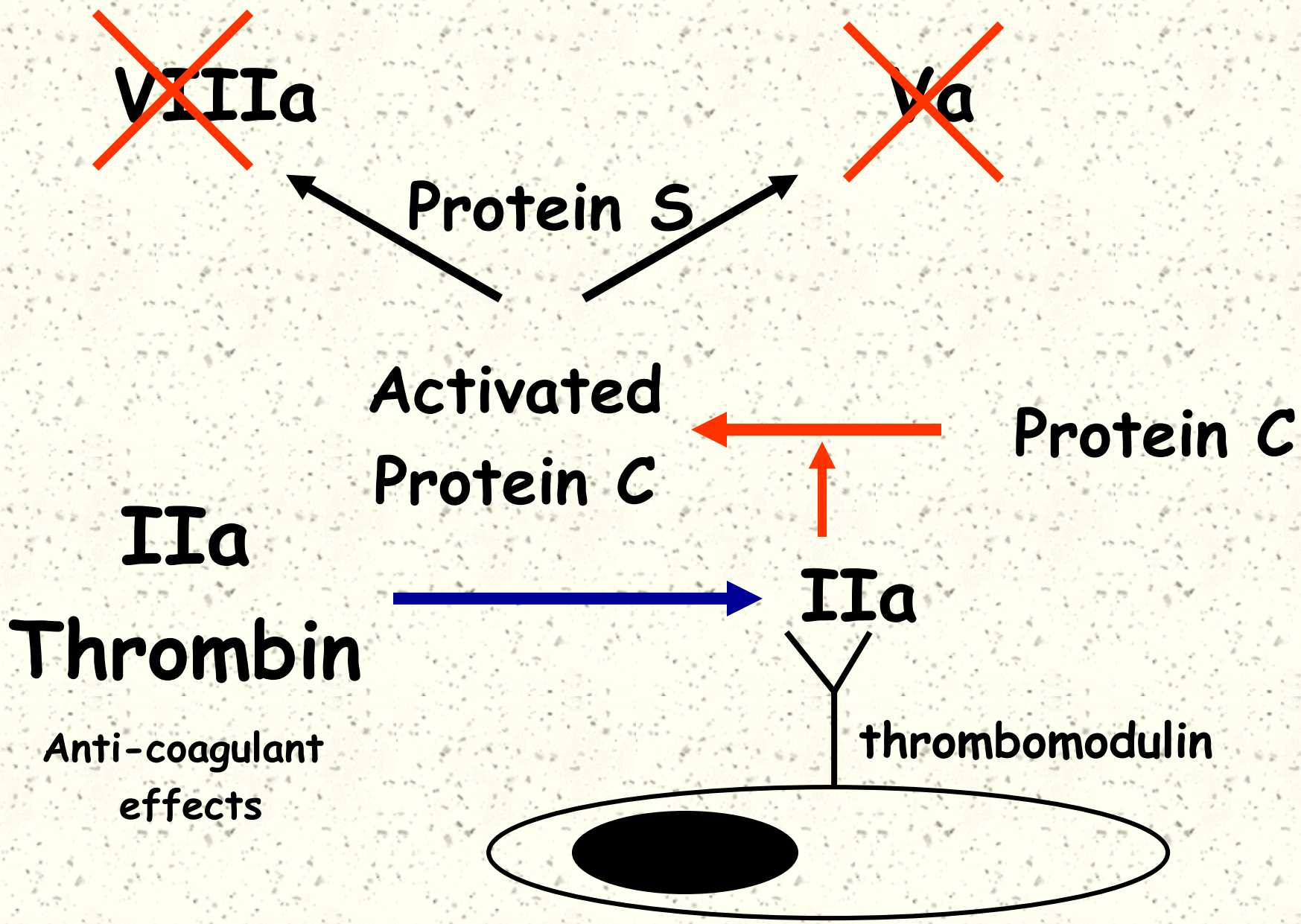



1. Severed vessel
2. Platelets agglutinate
3. Fibrin appears
4. Fibrin clot forms
5. Clot retraction occurs

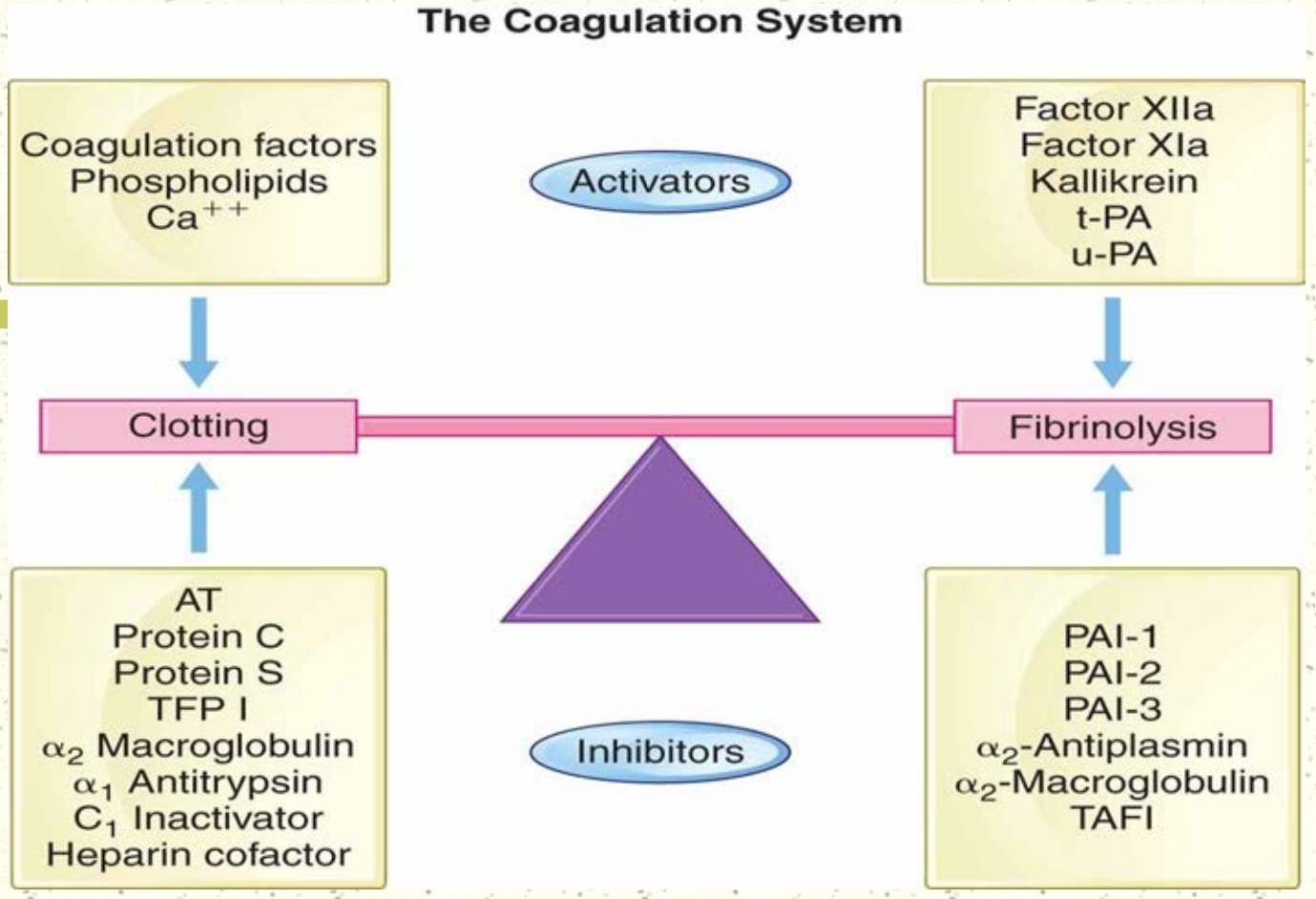
Coagulation Regulatory Mechanisms

- Naturally Occurring Anticoagulants rapidly interact with components of coag cascade to avoid unabated clot formation
  - Protein C (PC) and Protein S (PS)
    - deficiencies may be congenital or acquired
  - Antithrombin (AT) and Heparin Cofactor II
    - serine protease inhibitors (serpins)
- Deficiency of inhibitors cause increased risk of thrombosis


XII
XIIa
XI
XIa
IX
IXa
X
Xa
II
IIa
Protein S
Protein C
Protein S
VIIIa+Ca+Pl
Va+Ca+Pl
TFPI
TF / VIIa
IIa/Thrombomodulin interaction
Fibrinogen
Fibrin
Fibrinolysis

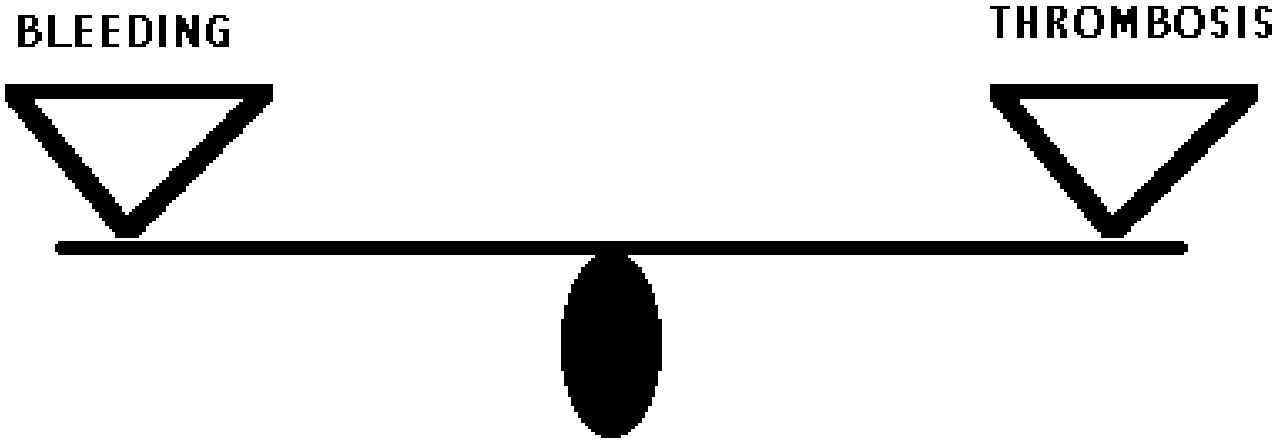


Protein C Anticoagulant Pathway



Coagulation system is kept in balance by activators and inhibitors of clotting and fibrinolysis.

Clotting occurs when blood vessels are damaged and activators of coagulation factors are released. Clotting is controlled by fibrinolysis. Inhibitors serve to bring the system back into balance.



- Without this balance, the individual may experience either excessive bleeding (poor clot formation or excessive Fibrinolysis)

- Vaso-occlusion (uncontrolled formation of thrombin in vascular system, occluding vessels and depriving organs of blood).

Tests of Hemostasis

- **Screening tests:**
  - Bleeding.T → 10m. Platelet & BV function
  - Prothrombin.T → Extrinsic, aPTT → Instrinsic
  - Thrombin.T → common path. (DIC)
- **Specific tests:**
  - Factor assays – hemophilia.
  - Tests of thrombosis – TT, FDP, DDA,
  - Platelet function studies:
    - Adhesion, Aggregation, Release tests.
  - Bone Marrow study

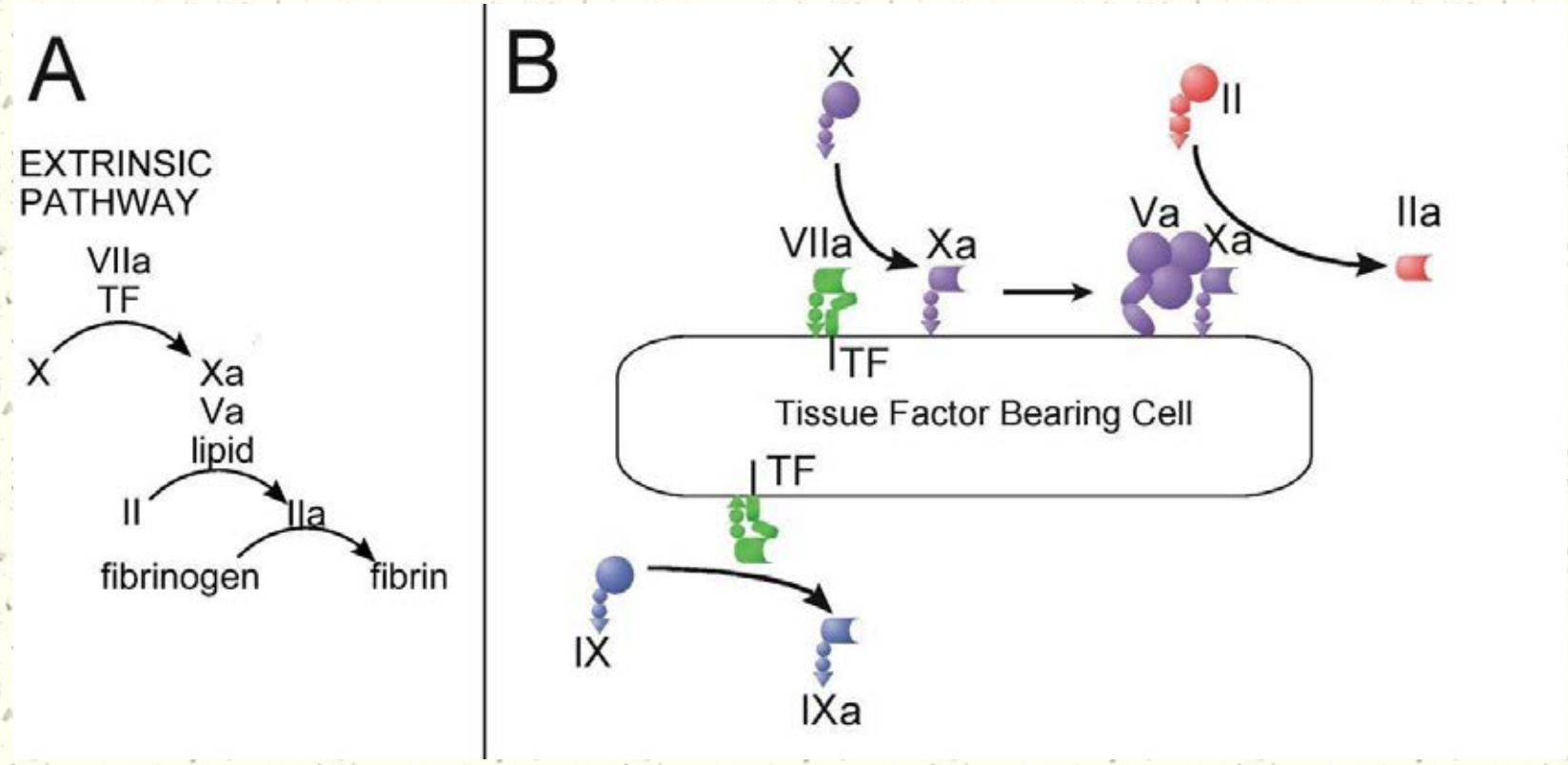
Blood coagulation tests

- **Bleeding time**
  - After incision to fingertip or earlobe – 1-6 min
- **Clotting time**
  - Blood colection in glass tube, invert tube every 30 s – wait until it clots (usually 6 min)
  - **Not reliable – not used clinically**

Prothrombin Time

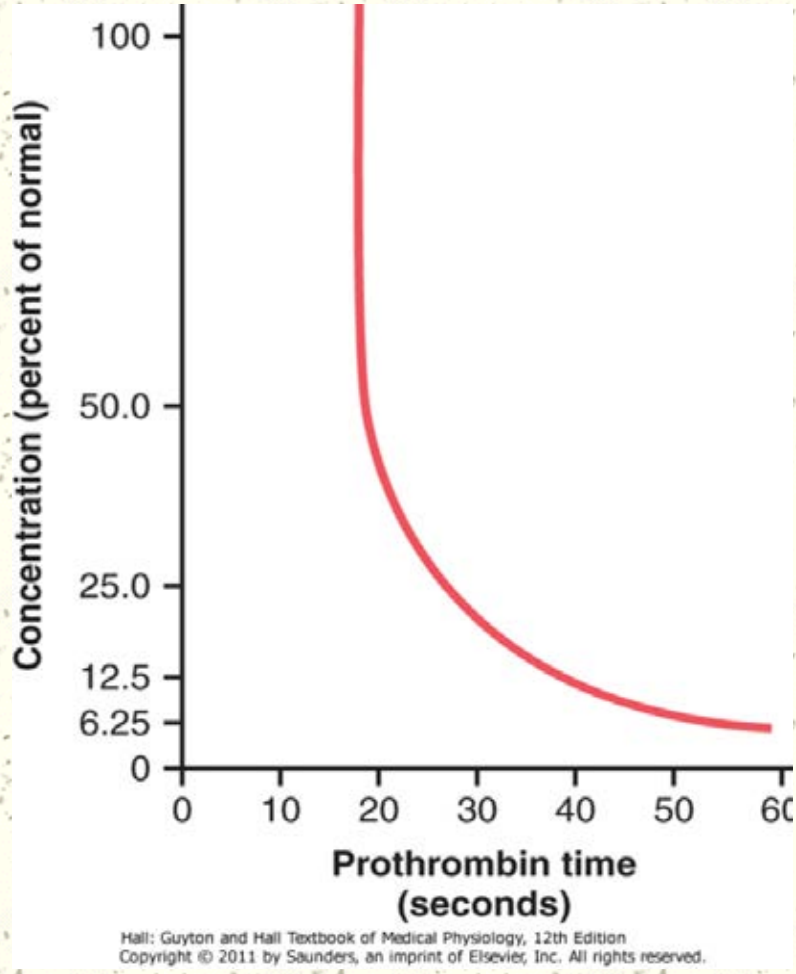
- Mixture of:
  - 50% patient's **platelet poor plasma**
  - 25% Mixture of **Tissue Factor** & **phospholipid species**
  - 25% **Calcium** chloride (to bring final calcium concentration to c. 3-5 mM)
  - **Time to clot formation measured**

PT
Extrinsic/Initiation pathway



Prothrombin Time

- Relates to concentration of prothrombin in patient's blood
- Oxalated blood given large amounts of Ca^{2+} and tissue factor – time to form a clot depends on the amound of prothrombin
- Usually 12 sec(**Normal Control)**
- Unreliable without normalization **(INR)** due to variability of effectiveness of tissue factor



Prothrombin Time

- **International normalized ratio**
  - Clinically used
  - Takes into account **international sensitivity index** *(ISI*: **1,0-2,0)** for each tissue factor batch
  - Normal INR: 0,9-1,3

$$INR = \left(\frac{PT_{test}}{PT_{normal}}\right)^{ISI}$$

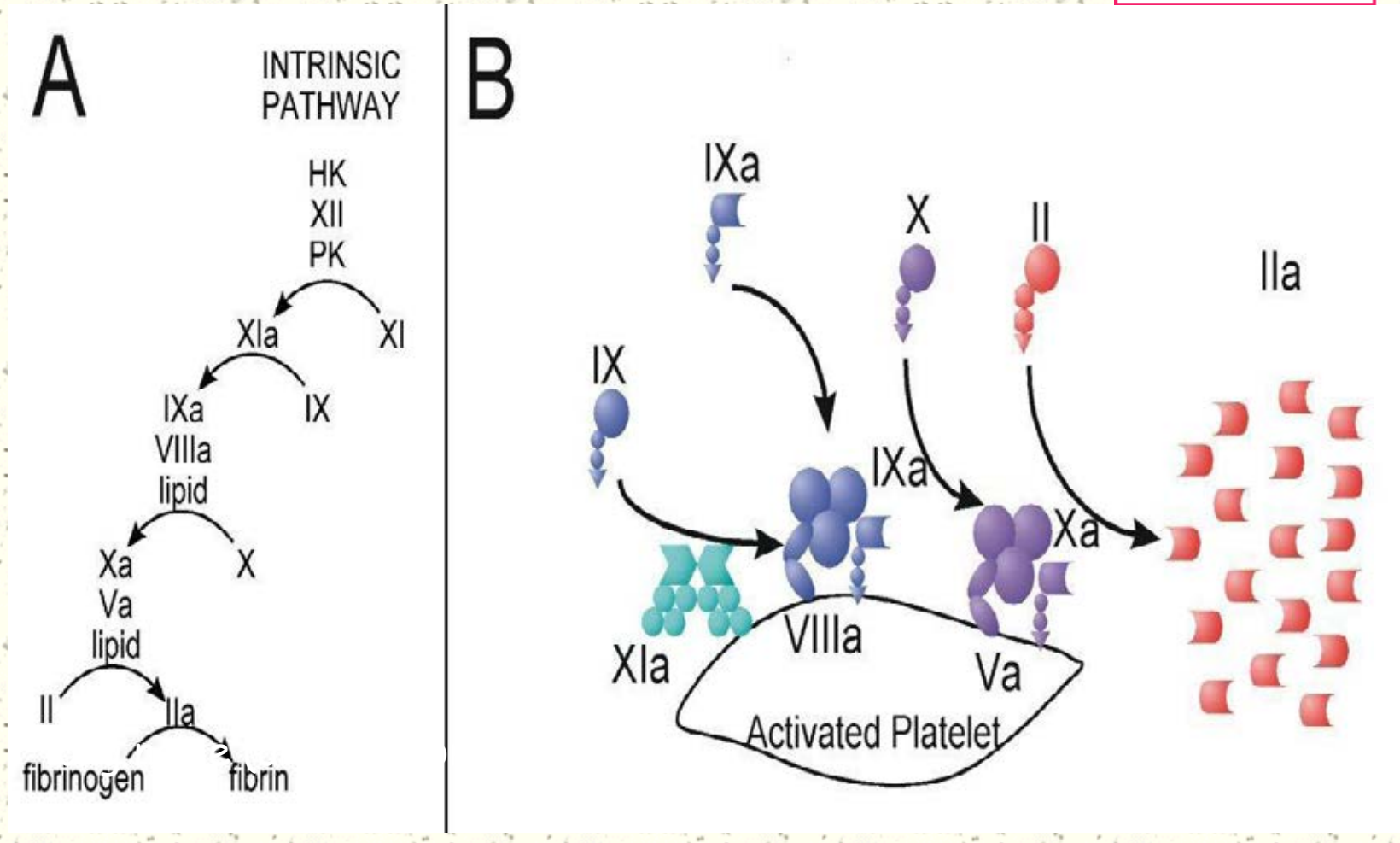
  - High INR:
    - 4-5 (high risk for bleeding)
    - 2,0-3,0 (warfarin therapy)

aPTT (activated partial thromboplastin time)

- 50% patient's **platelet-poor plasma**
- 25% mixture of **phospholipid & surface active agent** (Celite, Kaolin)
- 25% **Calcium** Chloride to bring calcium concentration to 3-5 mM
- **Time to clot formation measured**

aPTT
Intrinsic/Platelet pathway

aPTT measures the pathway that happens on the platelet surface



تطبيقات لاختبارات الارقاء
1 استقصاء خطر نزف

ا واب :

- البحث عن سوابق نزف شخصية أو عائلية
- نزف ولادي (نزف حبل سري، نزف طمثي، رعاف، كدمات عفوية، نزف بعد تداخل جراحي بسيط او قلع ضرس)
- قيك غير كافية او غير ممكنة (مريض غير واضح، طفل ل ذلك) خت بارات الشخيص ذات نزفي

تطبيقات اختبارات الارقاء

2- اختبارات الارقاء في تشخيص النزف

- تعداد الصفيحات، زمن كويك (PT)، الزمن الجزئي للثرومبوبلاستين المفعل (aPTT)

والتحاليل الرئيسية التي تقدمها هذه الاختبارات هي:

- **نقص الصفيحات:** تتبع نقص كاذب
  - اما مركزي (خلل في النقي)
  - أو محيطي نتيجة تخرب الصفيحات كما
- **aPTT طبيعي و PT متطاول:**
  - عوز عامل FVII وراثي او مكتسب
- **aPTT متطاول و PT طبيعي:**
  - ناعور A او B
  - مرض فون ويلبراند (غالبا نقص FVIII)
  - عوز FXI (نزف في 1/3 الحالات)
  - باب لا يرى تطاول PT او aPTT غير نزف عادة :
    - اضطراب نظام التماس
    - اضداد تخثر جوالة ذئبية

ALLONGEMENT ISOLE

du TCA

TCA ↗ TQ = Nl

- Déficit Phase Contact:
  - F XII
  - F XI
  - Prékallicréine
  - KHPM
- Hémophilies
  - Déficit F VIII
  - Déficit F IX
- Lupus anticoagulant

du TQ

TQ ↗ TCA = Nl

- Déficit F VII

تطبيقات اختبارات الارقاء

2- اختبارات الارقاء لتشخيص النزف

- PTT و PT متطاول :
- اضطراب فيبرينوجين: <1g/L
  - اما (غياب، نقص، عدم الفعالية)
  - او مكتسب (نخر كبدي، تخثر منتشر داخل الاوعية)
- يجب ضبط عيار عوامل البروثرومبين: I,II,V,X في حالة نقص معزول او مشترك في قصور الخلايا الكبدية او نقص Vit k
- اما النزف في وجود تحاليل طبيعية يجب اجراء PT,PTT,FvW,FVIII :
  - في 10-20% من مرضى فون ويلبراند يكون PTT طبيعيا
  - اذا كان FvW طبيعي يجب اجراء اختبارات وظائف الصفيحات (النزف الصفيحي)

TCA ↑ + ↑ TQ

الفيبرينوجين

الفيبرينوجين ≤ 1غ/ل

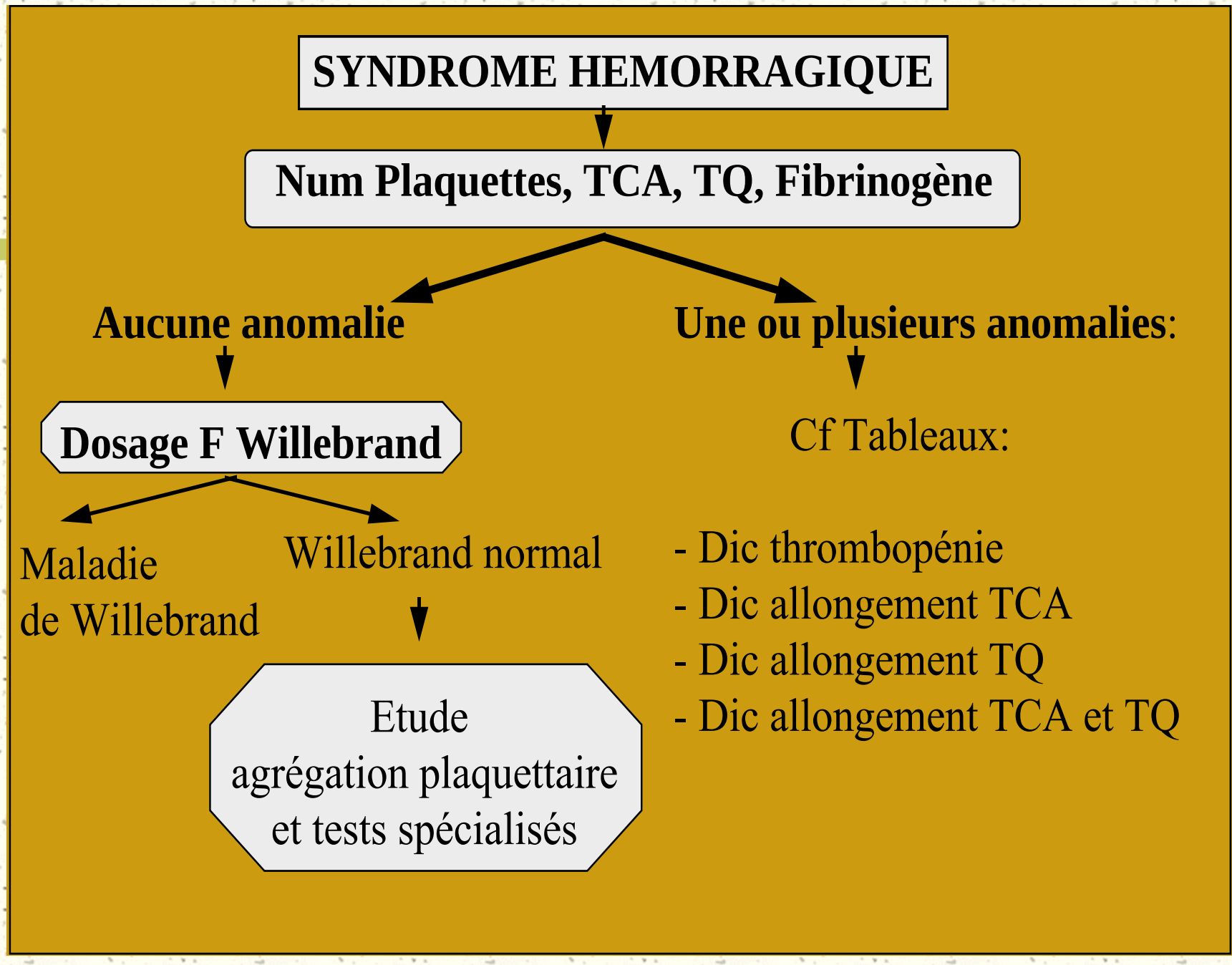
الفيبرينوجين > 1 غ/ل

عيار عوامل مركب البروترومبين

D-Dimers، TLE، الصفيحات،

- نقص في عامل V,X,VII و II= قصور خلايا الكبد (قصور في تركيب البروتينات)
- نقص عامل II,X,VII بينما العامل V طبيعي= عوز فيتامين ك
- أعراض معزول في العامل X,V,II (+ العامل VII)

- نقص معزول للفيبرينوجين: انعدام، نقص او عسر فيبرينوجين
- نقص الصفيحات، D-Dimers ++++++, CS+ = DIC
- الصفيحات ط، D-Di +++ قصر TLE = انحلال ليفي بدائي


SYNDROME HEMORRAGIQUE
Num Plaquettes, TCA, TQ, Fibrinogène
Aucune anomalie
Une ou plusieurs anomalies:
Dosage F Willebrand
Cf Tableaux:
Maladie de Willebrand
Willebrand normal
- Dic thrombopénie
- Dic allongement TCA
- Dic allongement TQ
- Dic allongement TCA et TQ
Etude agrégation plaquettaire et tests spécialisés

الاجراءات والعلاقات التي يجب تجنبها عند تعرض الموظف للابتزاز


THANK YOU

Platelet Aggregation