بسم الله الرحمن الرحیم

هست کلید در گنج حکیم

Hemostasis & disorders

Coagulation = Love !!!!

Every body **talk** about it,

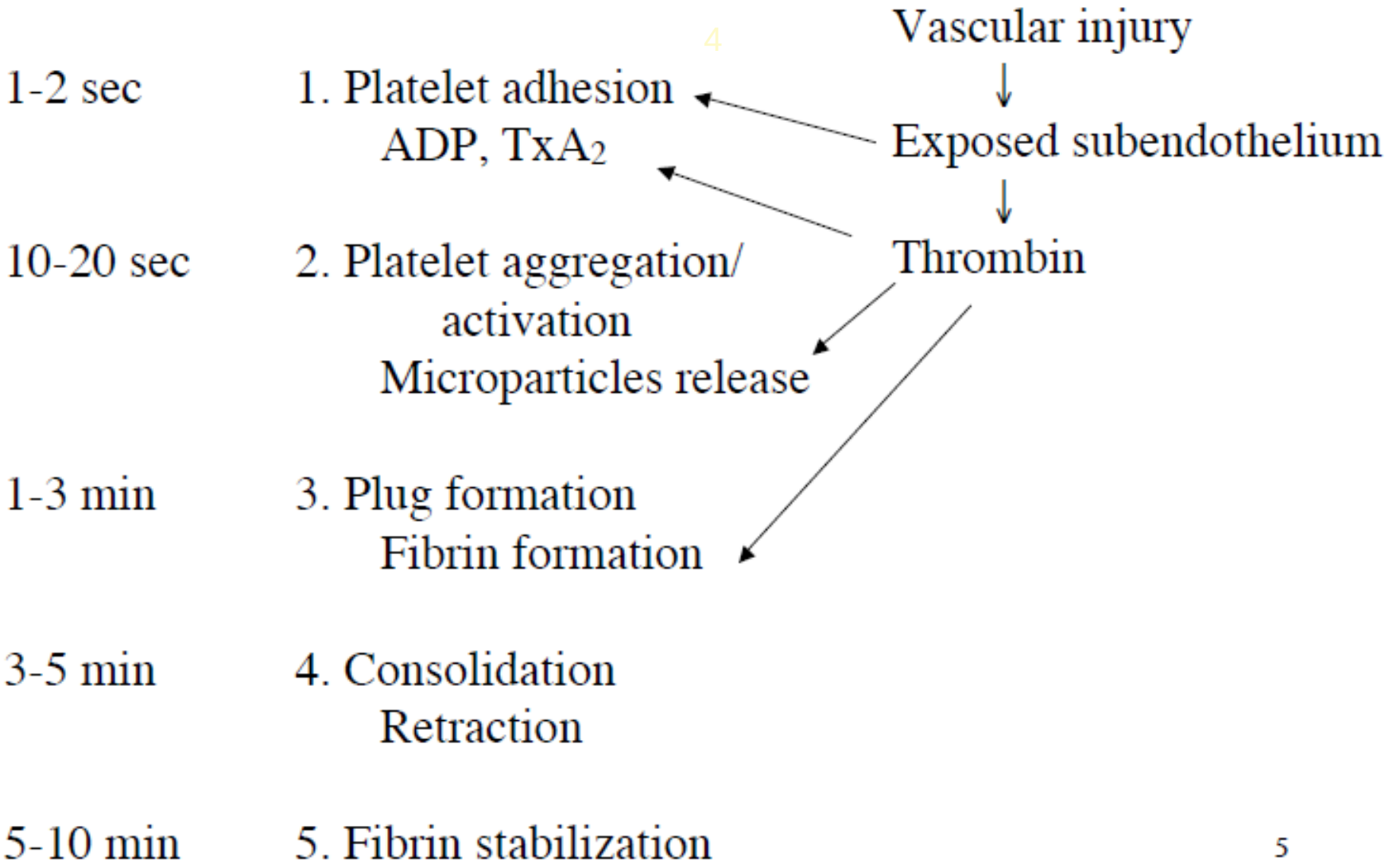
But

Just a few people **understand** it

Hemostasis

- *Heme=* blood
- *stasis=* to halt
- Process of retaining blood within the vascular system
- Repairs injury to blood vessels
- Stops or prevents blood loss

Overview of Hemostasis



Hemostasis

- Components
  - Vascular System
    - Controls rate of blood flow
  - Platelet System
    - Interaction of vasculature and platelets form a **temporary** plug
  - Coagulation System
    - (i.e) fibrin forming
  - Fibrinolytic System
    - Fibrin lysing
  - Coagulation Inhibition System
    - Natural inhibitors
    - Control fibrin formation and fibrin lysis

Failure or deficiencies in any of these five systems can leads to varying degrees of uncontrolled hemorrhaging or clotting

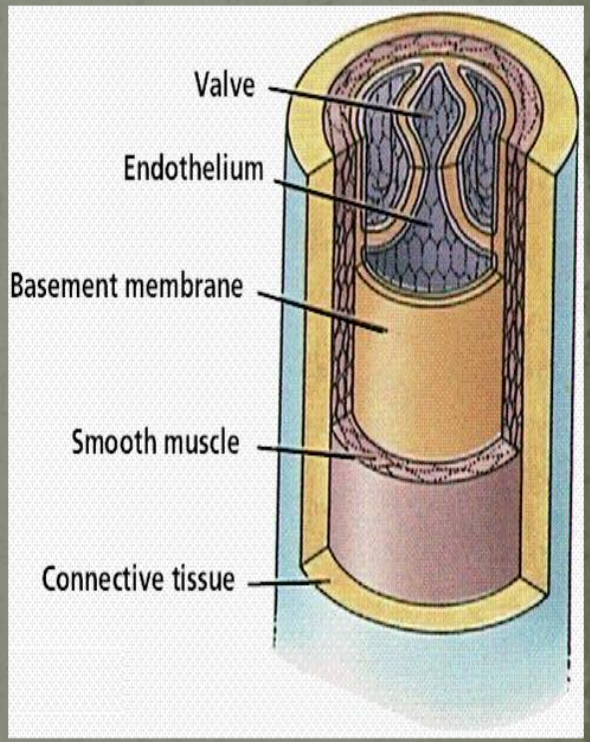
Following injury, each component must function optimally.

Hemostasis Consists of three stages

- Primary Hemostasis
  - Process of blood clotting in response to injury where blood vessels (vasculature) and platelets and vWF are the main "players."
  - **Primary** Hemostatic **plug** is formed
- Secondary Hemostasis
  - Actions of the coagulation factors in response to injury
  - At this time, blood has changed into a solid state
- Fibrinolysis
  - Clot is removed following healing of wound

Vascular System: Blood Vessels

- Endothelium
  - Single layer of endothelial cells, lining vessels
  - Coated by glycocalyx
  - Produces Von Willebrand's factor (vWF)
  - Secretes prostaglandins, plasminogen activators
  - Negatively charged, repels circulating proteins and platelets
- Subendothelium
  - Smooth muscle and connective tissue with collagen fibers
- Basement membrane
  - Collagen material – stimulates platelets
- Connective tissue
  - Elastic fibers- provide support around vessels



Vascular System: Function Following Injury

- Initiate hemostasis
  - Vasoconstriction of the arterioles
    - Minimizes blood flow to injured area
    - Prevents blood loss
    - Immediate
    - Short-lived

Platelets:

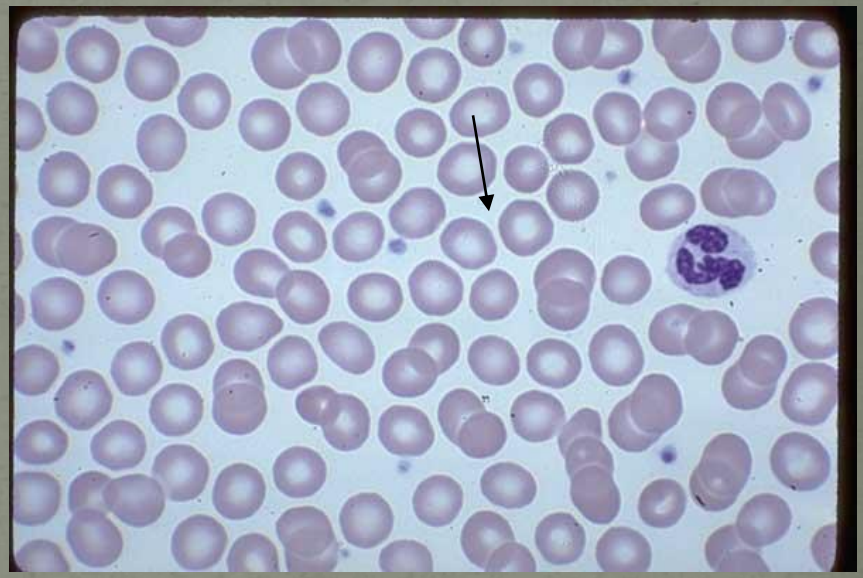
Second major component of the hemostatic system

- Small 2-3 μm
- Anuclear
- Fragments of megakaryocyte cytoplasm

- Life span
  - 9-10 days
- Normal Range
  - 150-450 x 10^9 /L

Growth factors:

TPO and IL-11



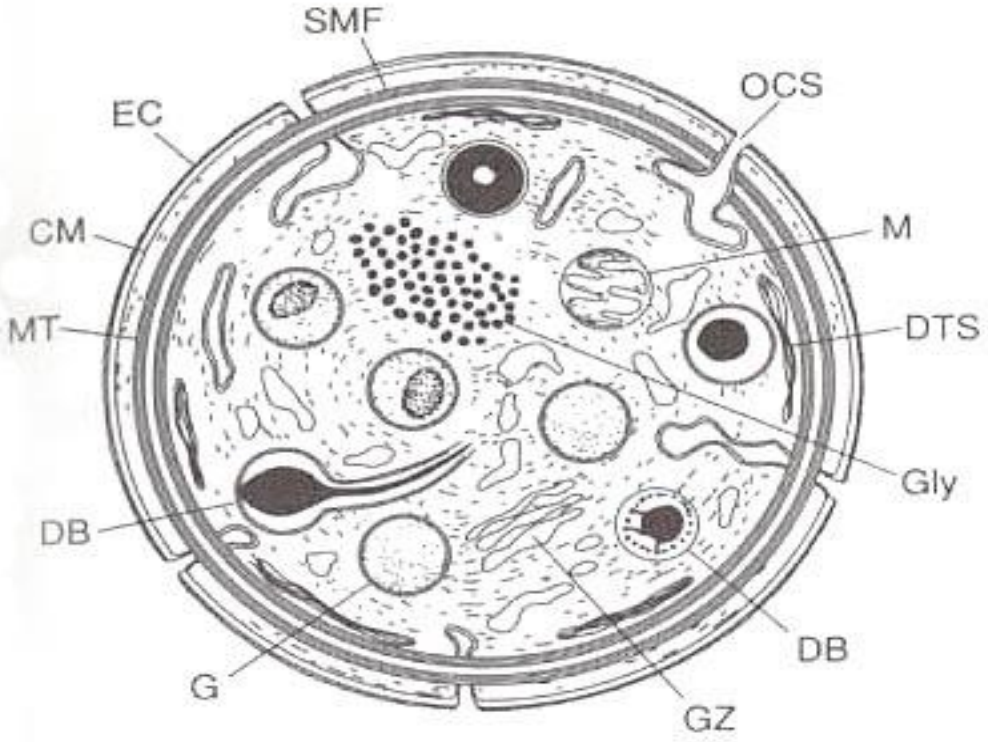
Anatomy of a Platelet

- **Peripheral zone**: Responsible for platelet adhesion and aggregation
  - ***Glycocalyx***:
    - Contains glycoprotein receptors:
      - **GPIb** binds von Willebrand's factor needed for platelet adhesion to collagen
      - **GPIIb/IIIa** bind fibrinogen needed for aggregation
  - ***Plasma membrane:***
    - Exposed on platelet activation
      - Layer called **PF3** (platelet factor) surface for interaction of plasma coagulation factors
      - Initiation of **formation of thromboxane A_{2} ($Tx\ A_{2}$) . that** stimulates aggregation and vasoconstriction

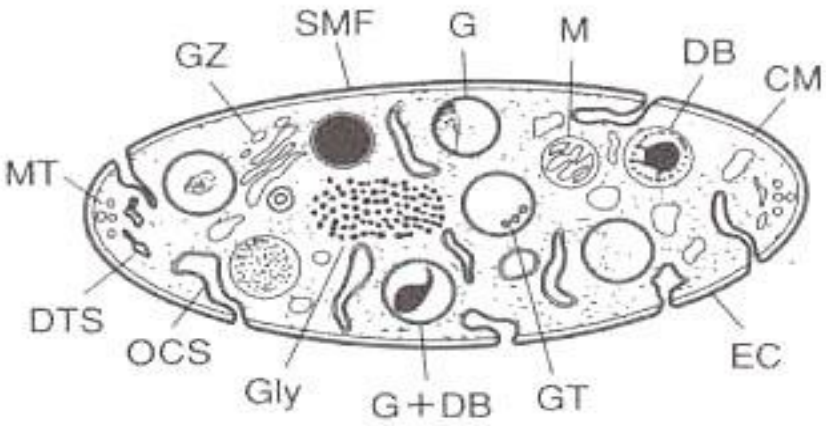
Anatomy of a Platelet

- **Structural or Sol-Gel zone:** Responsible for platelet retraction/contraction functions and platelet shape
  - Microtubules
  - Cytoskeleton
  - Binding protein

- **Organelle zone:** Responsible for storage and platelet release functions
  - Granules
    - Dense bodies, alpha granules, lysosomal granules and microperoxisomes
  - Mitochondria
  - Glycogen



Schematic figure for electron microscopic picture of equatorial section of platelet (White, 1971)



Electron microscopic picture of longitudinal section of platelet

EC : external coat (surface coat)
CM : cell membrane (unit membrane)
SMF : submembrane filaments
MT : microtubules
DB : dense body
G : α-granules (specific granules)

M : mitochondria
OCS : open canalicular system
DTS : dense tubular system
GZ : Golgi zone (Golgi apparatus)
Gly : Glycogen

Fig. 4 Captions for Photos 3 and 4

Platelet Receptors

- GPIb/IX – vWF
  - Required for PLT adhesion
- GPIIb/IIIa – Fibrinogen
  - Required for PLT aggregation
- Phospholipid (Pl)
  - Bind vitamin K dependent proteins , Ca^{++} dependent
  - Bind Va and VIIIa (called “PF_3” in this context)

Stage 1: Platelet Adhesion

stages of platelet activation and plug formation

Stage 2: Platelet Aggregation

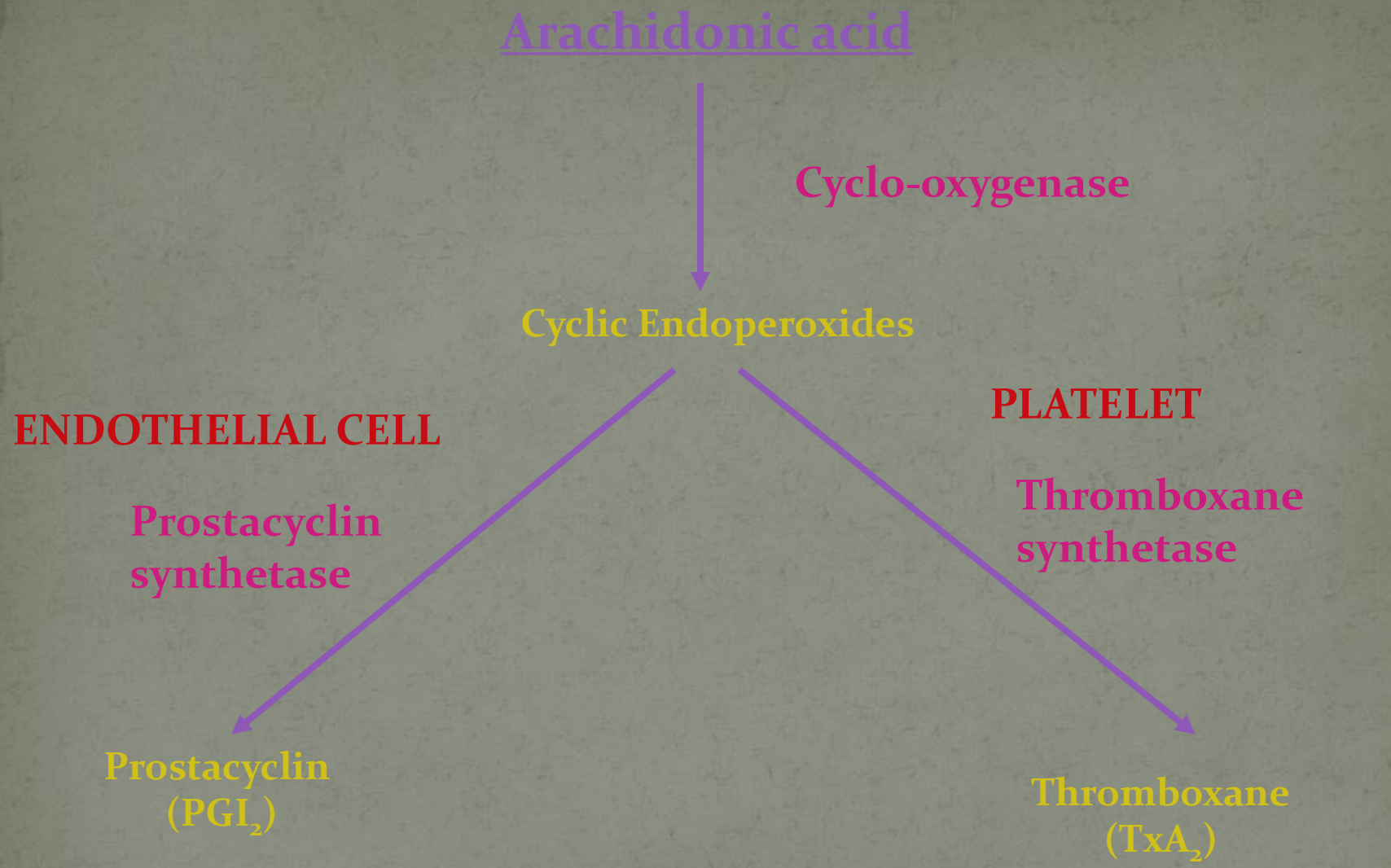
Stage 3: Platelet Secretion

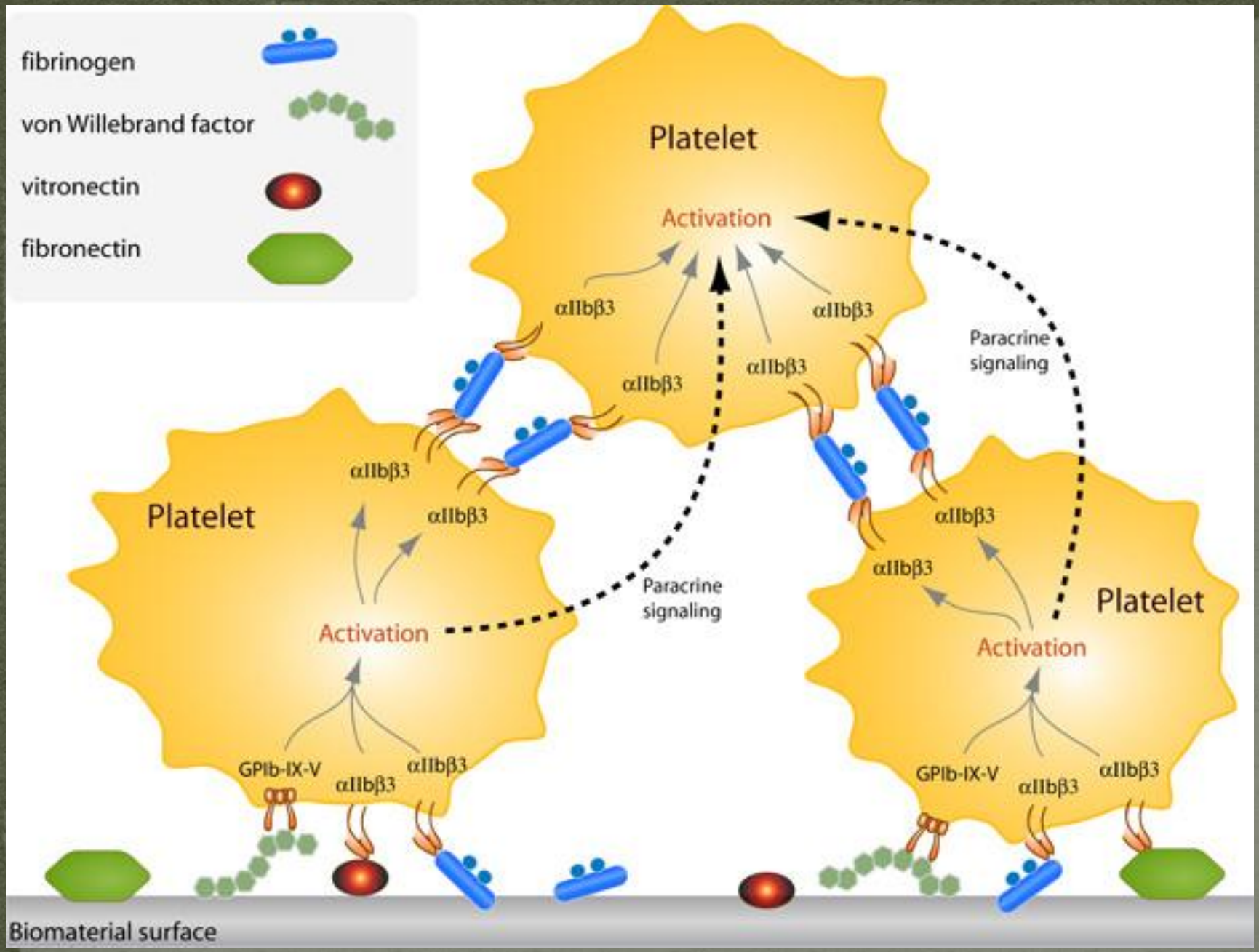
Stage 1: Platelet Adhesion

- Platelets attach to non-platelet surfaces, such as collagen fibers in the subendothelium
- Exposure to surfaces in the tissues causes them to bind to collagen with the presence of von Willebrand factor (vWF) and Glycoprotein IbIX, making a bridge formation, which triggers a shape change
- Reversible

Stage 2: Platelet aggregation

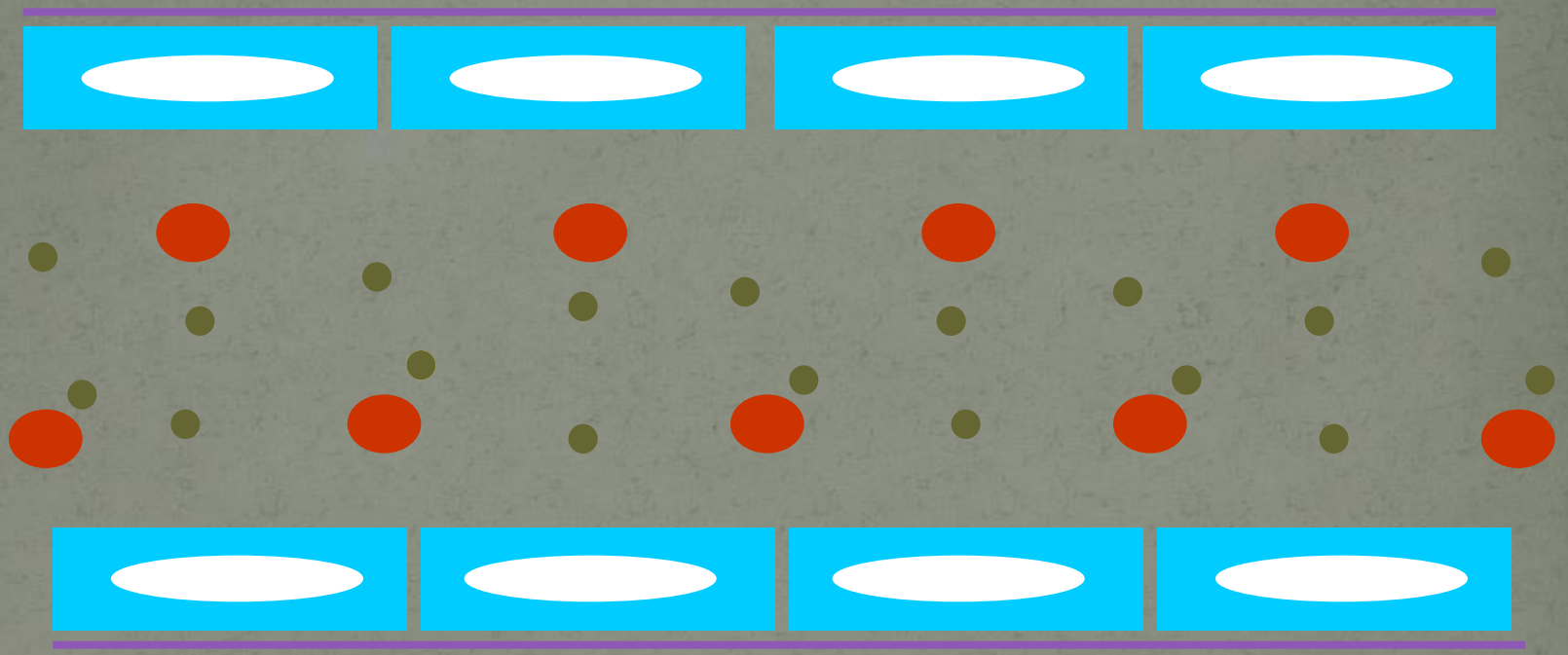
- Chemical changes cause platelets to aggregate and stick to one another
- Newly arriving platelets become activated by agonists
- Exposure of GPIIb/IIIa sites bind fibrinogen
- Fibrinogen + activated platelets serves as a bridge between two platelets
- Calcium must be present
- Activated platelet membrane generates TXA_2
- TXA_2 stimulates release

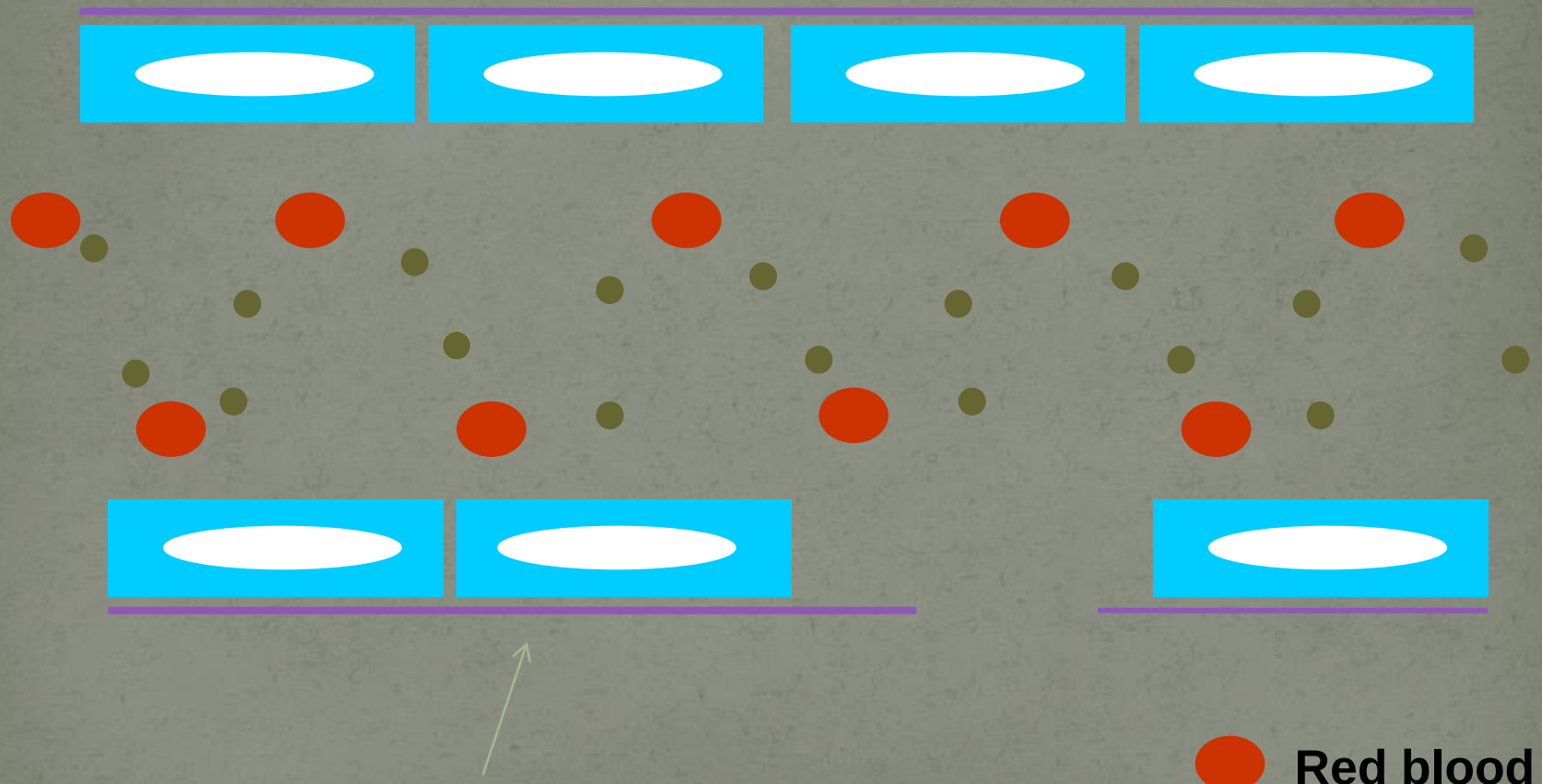

Arachidonic acid
Cyclo-oxygenase
Cyclic Endoperoxides
ENDOTHELIAL CELL
Prostacyclin synthetase
Prostacyclin (PGI_2)
PLATELET
Thromboxane synthetase
Thromboxane (TxA_2)
Inhibits plt aggregation
Vasodilator
Enhances plt aggregation
Vasoconstrictor

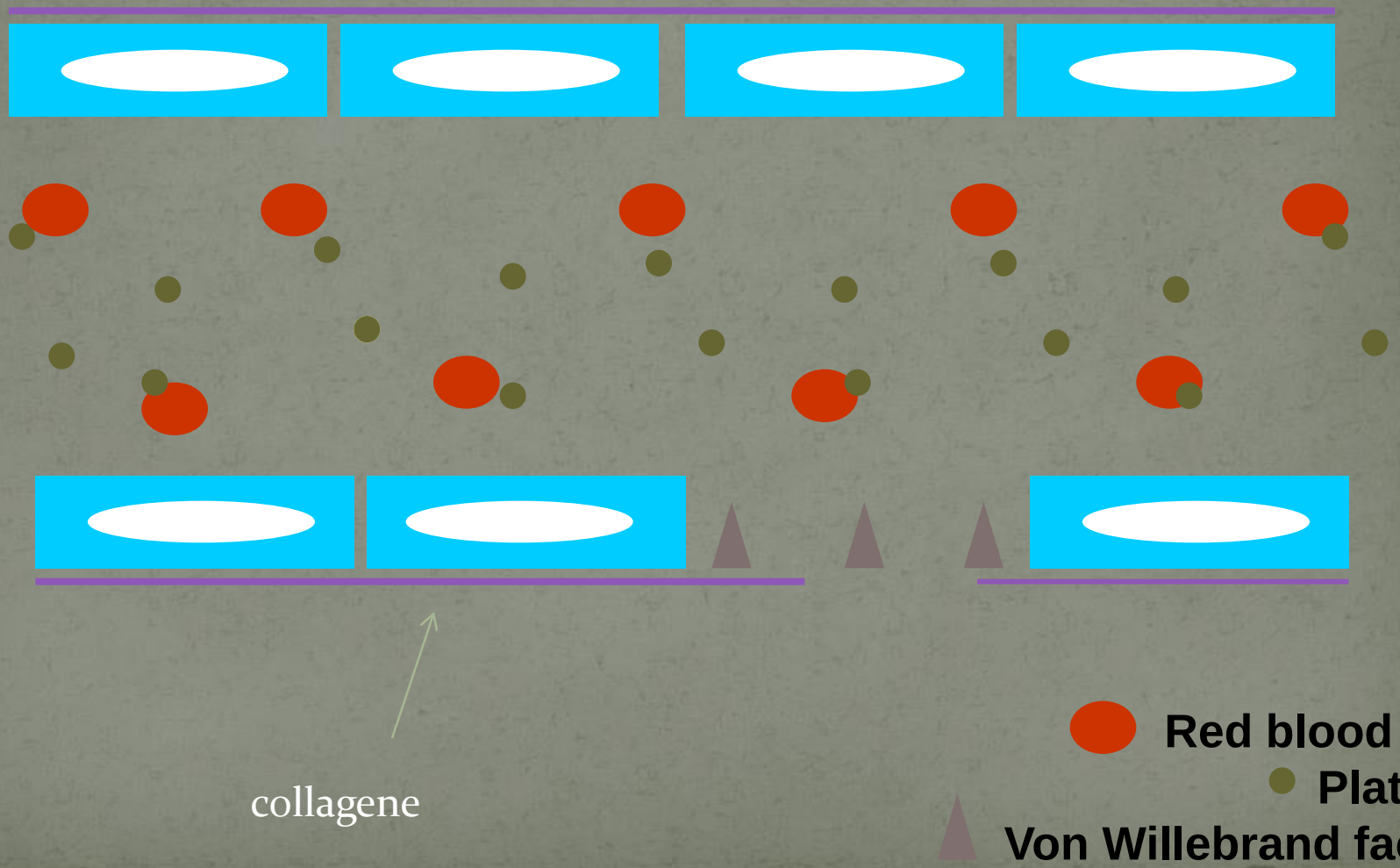

fibrinogen
von Willebrand factor
vitronectin
fibronectin
Platelet
Activation
αIIbβ3
αIIbβ3
αIIbβ3
αIIbβ3
Paracrine signaling
Platelet
αIIbβ3
αIIbβ3
Activation
Paracrine signaling
GPIb-IX-V
αIIbβ3
αIIbβ3
αIIbβ3
αIIbβ3
Platelet
Activation
GPIb-IX-V
αIIbβ3
αIIbβ3
Biomaterial surface

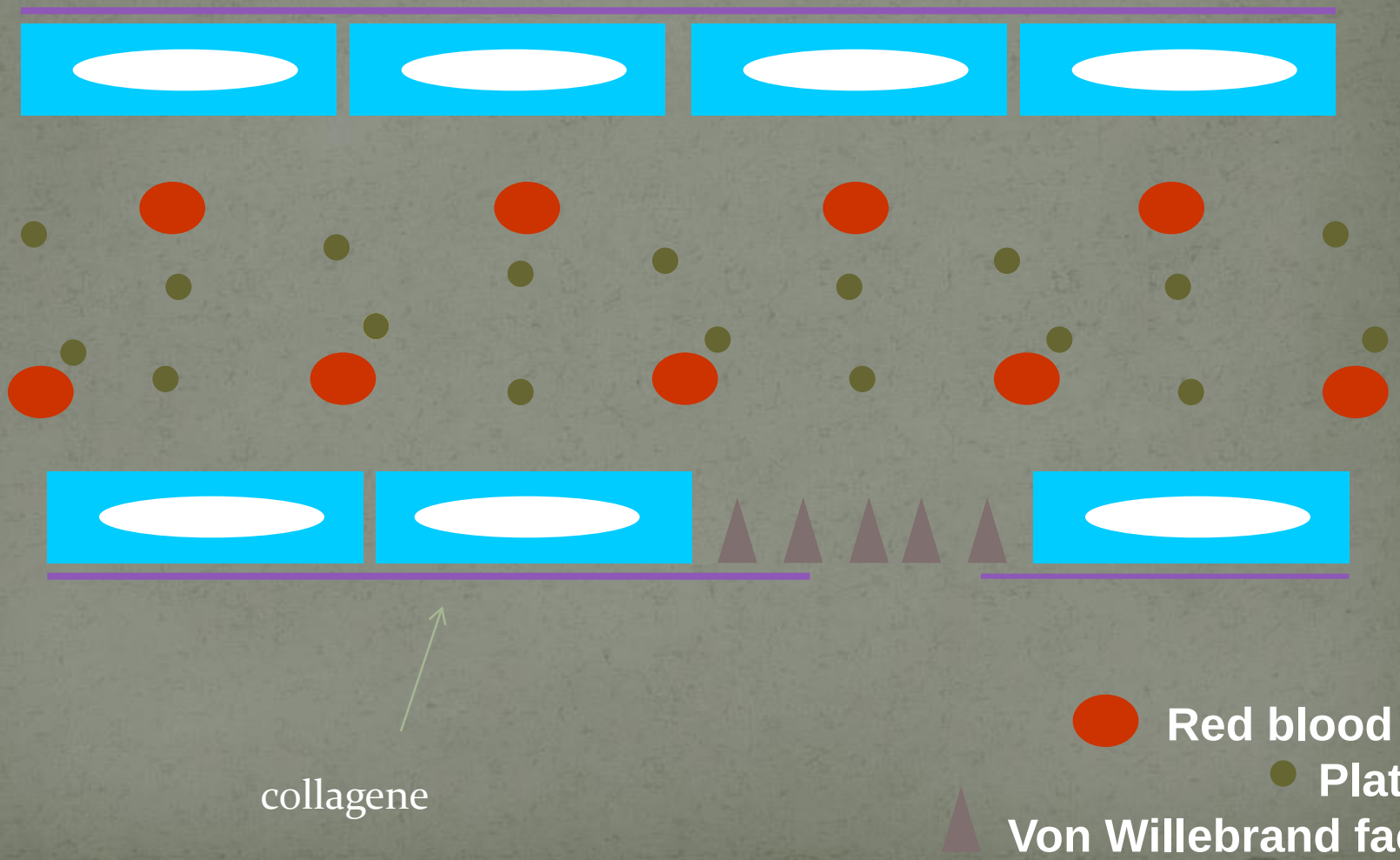
Stage 3: Platelet Secretion & Release

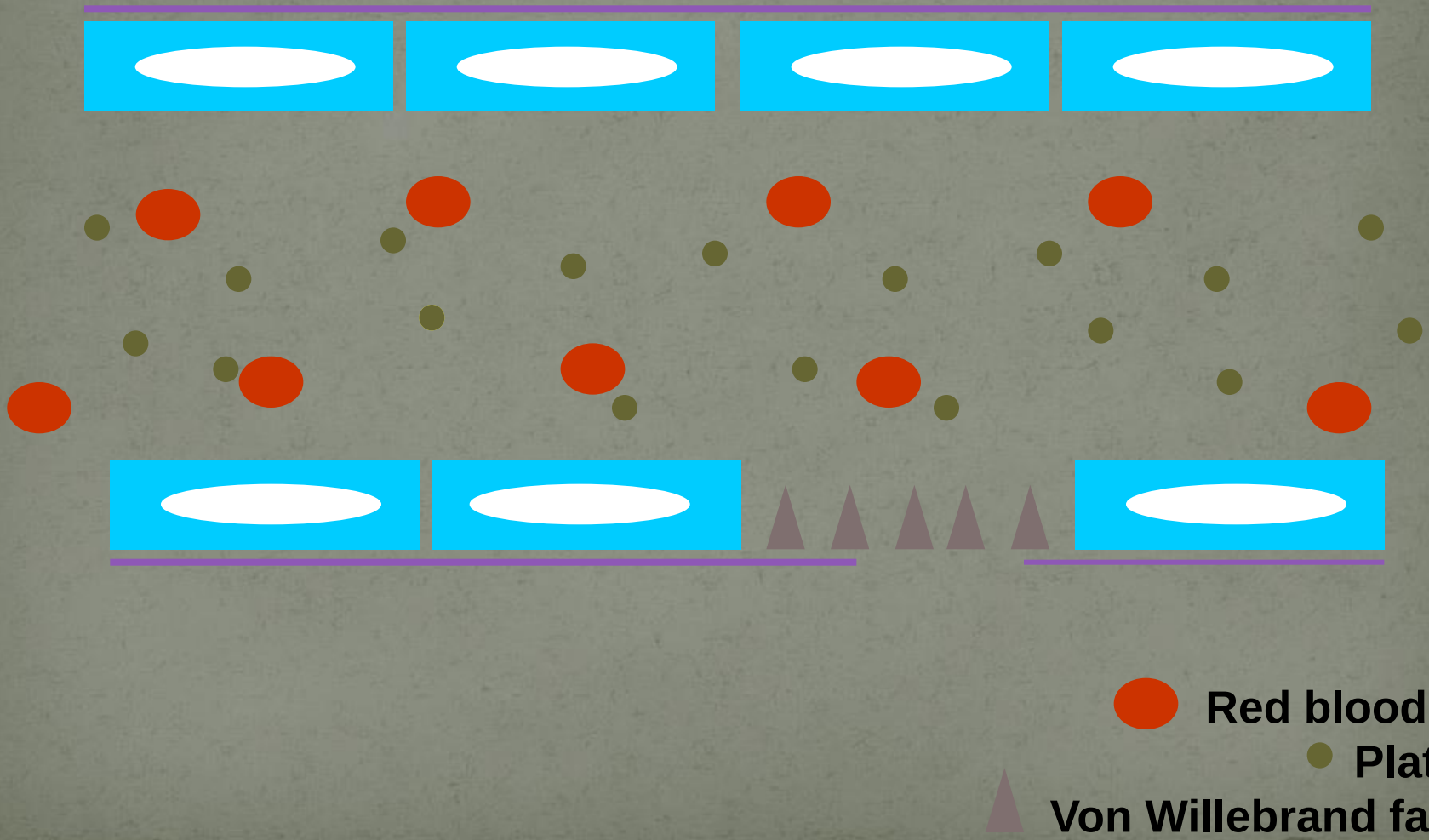
- Requires ATP
- Platelets release contents of their granules, causing vasoconstriction
- Granules trigger a secondary aggregation which is irreversible
- Granules consist of
  - Alpha granules: Factor V, Factor VIII:vWF, Fibrinogen, α2-antiplasmin, platelet factor 4
  - Dense bodies: ATP, ADP, serotonin, Ca

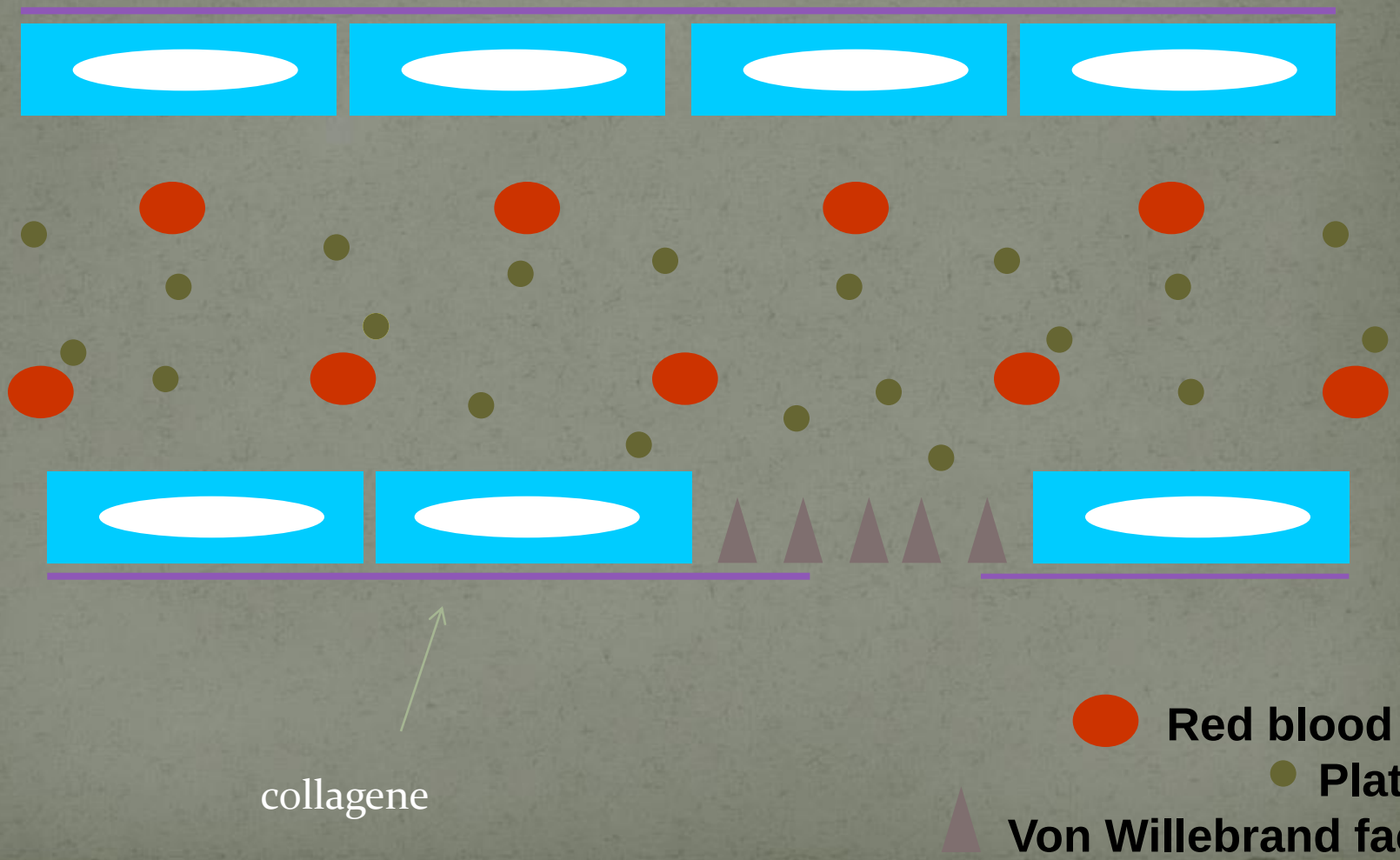

collagene
Red blood cell
Platelet

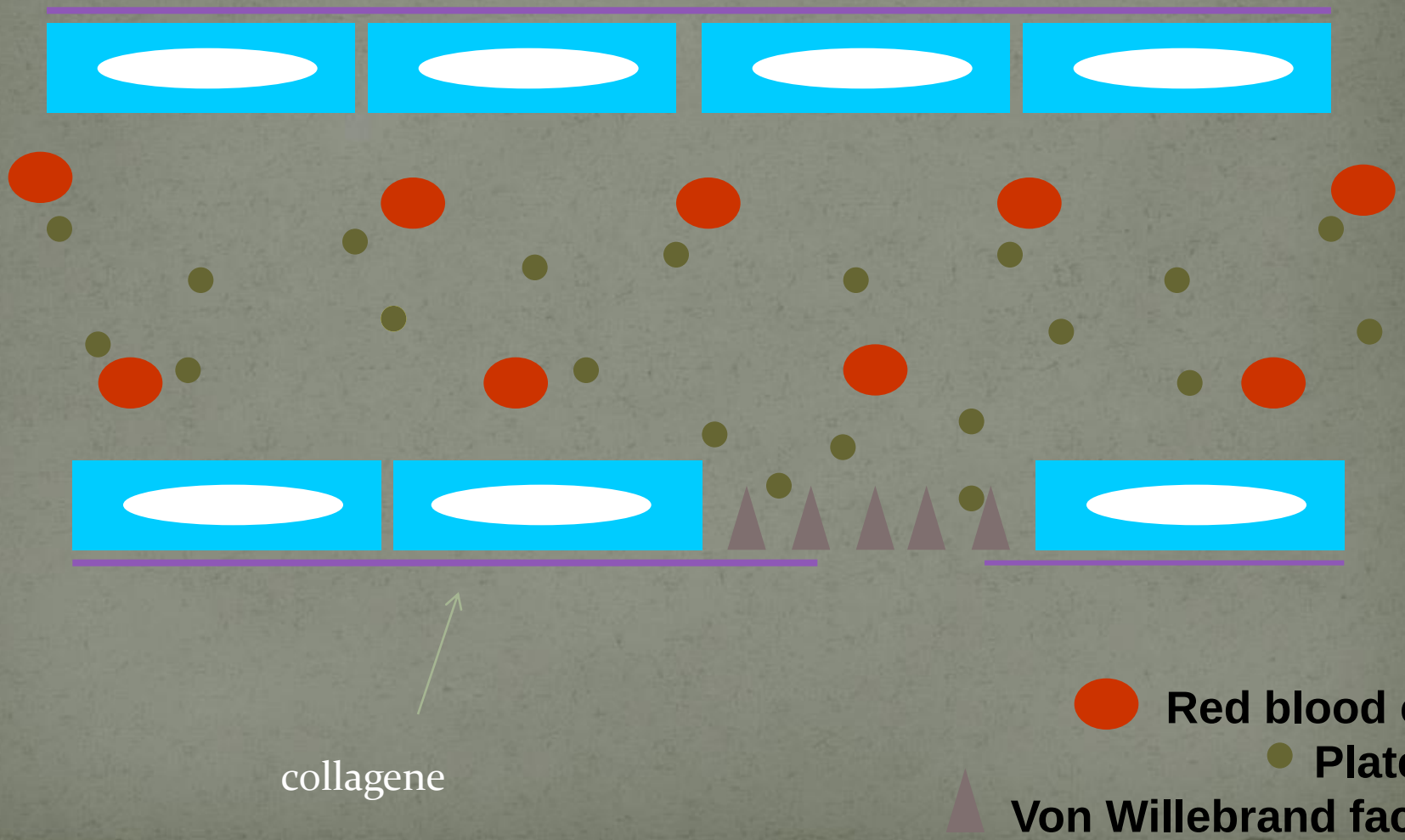

collagene
Red blood cell
Platelet

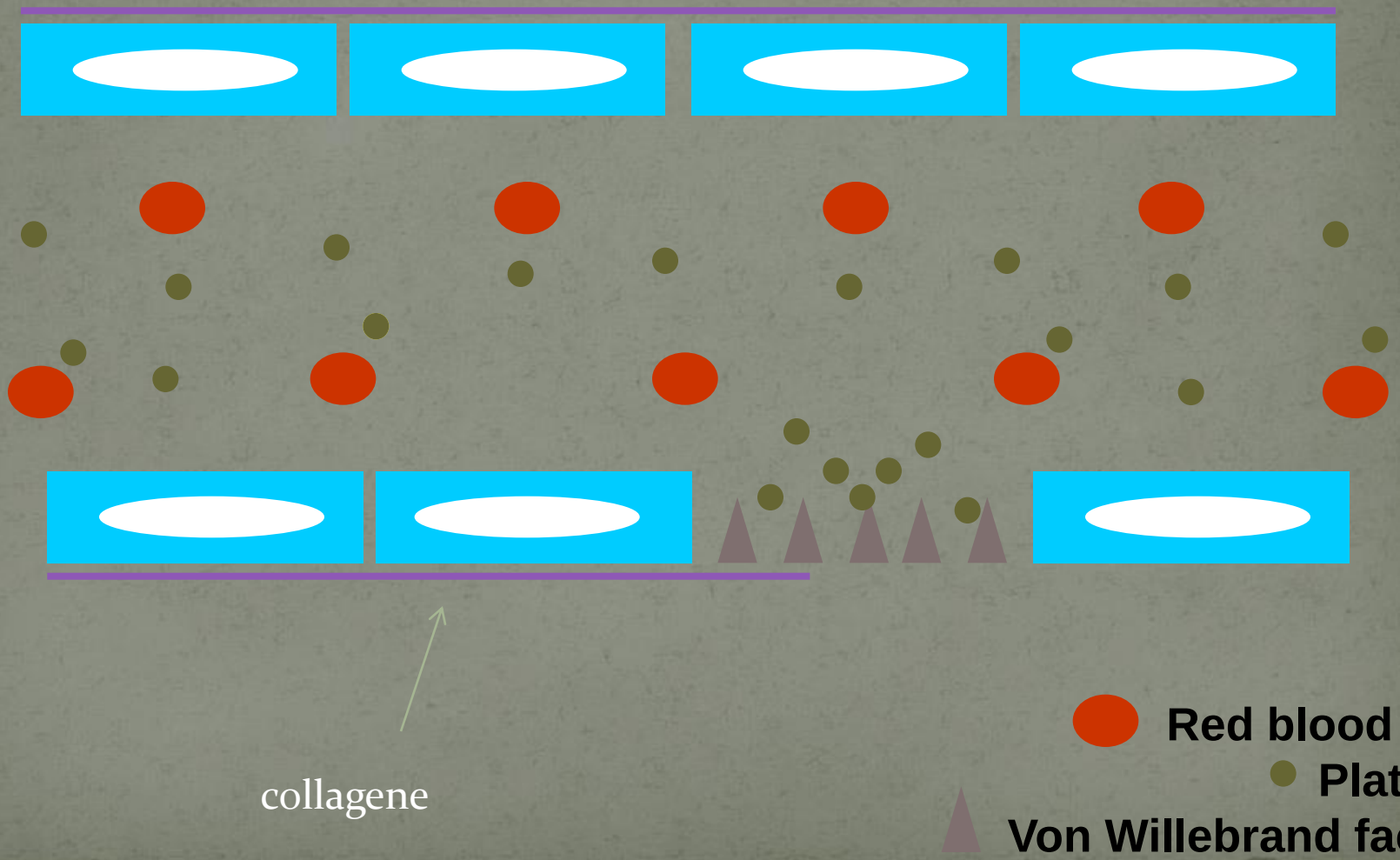

collagene
Red blood cell
Platelet
Von Willebrand factor

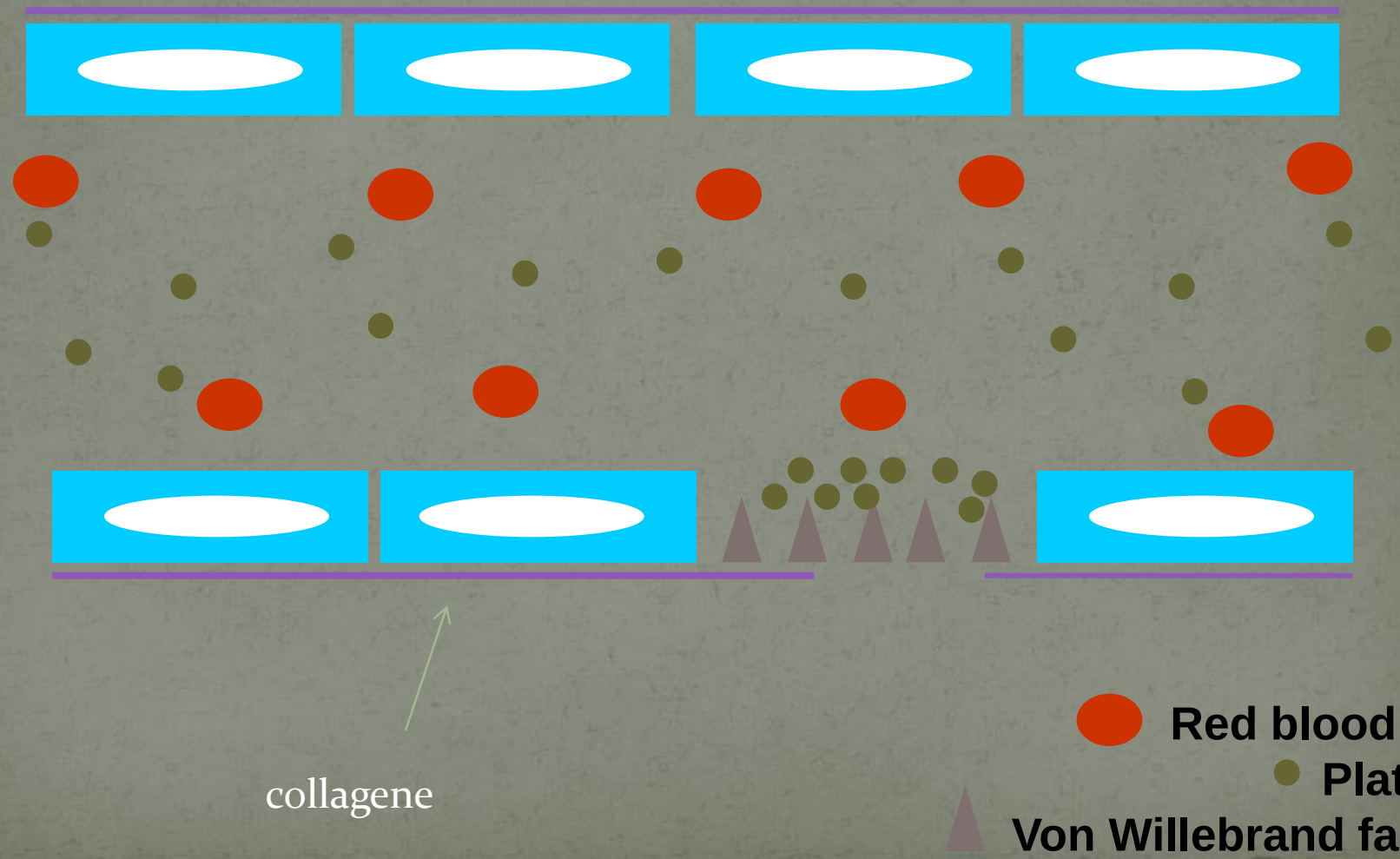

collagene
Red blood cell
Platelet
Von Willebrand factor

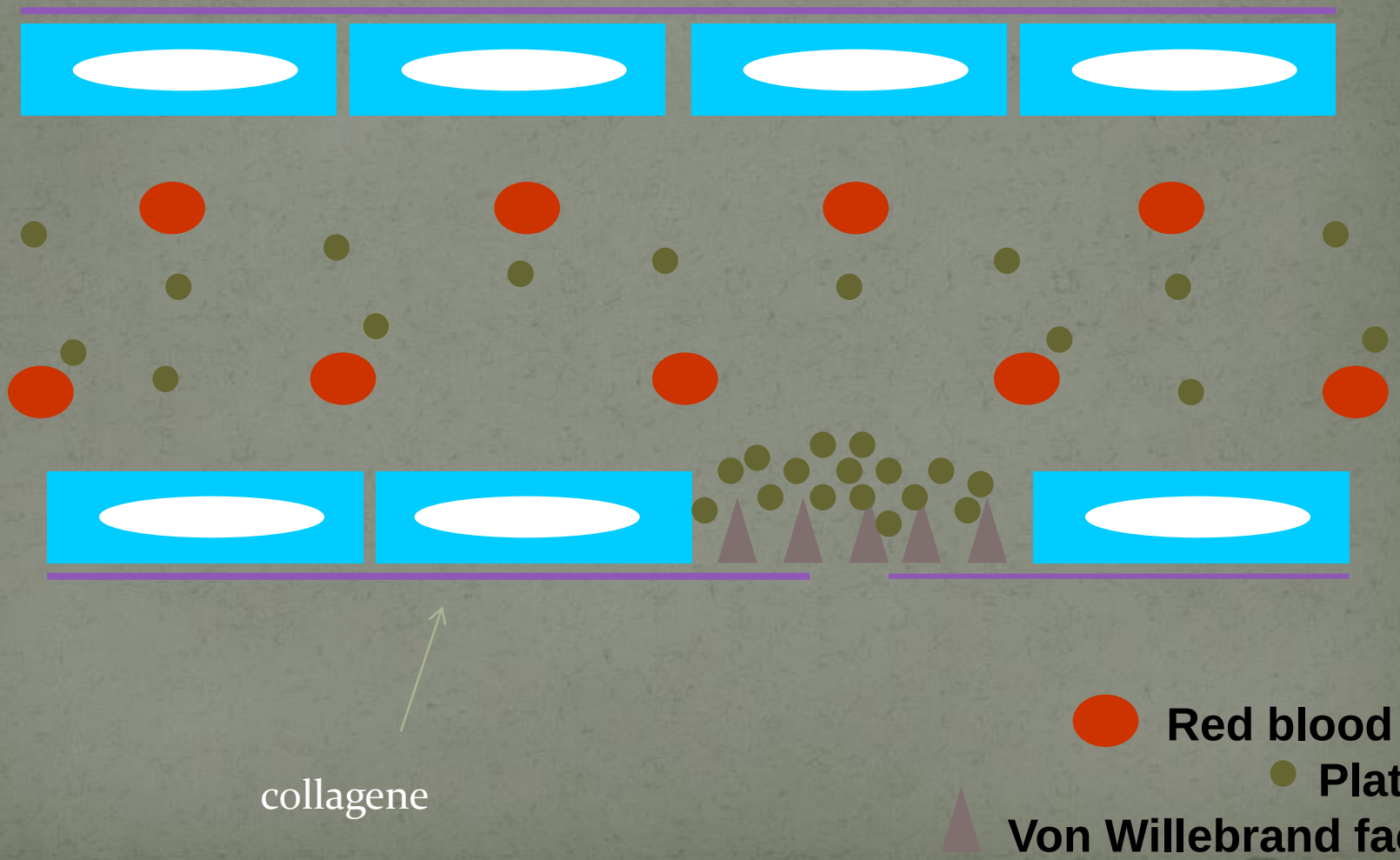

Red blood cell
Platelet
Von Willebrand factor

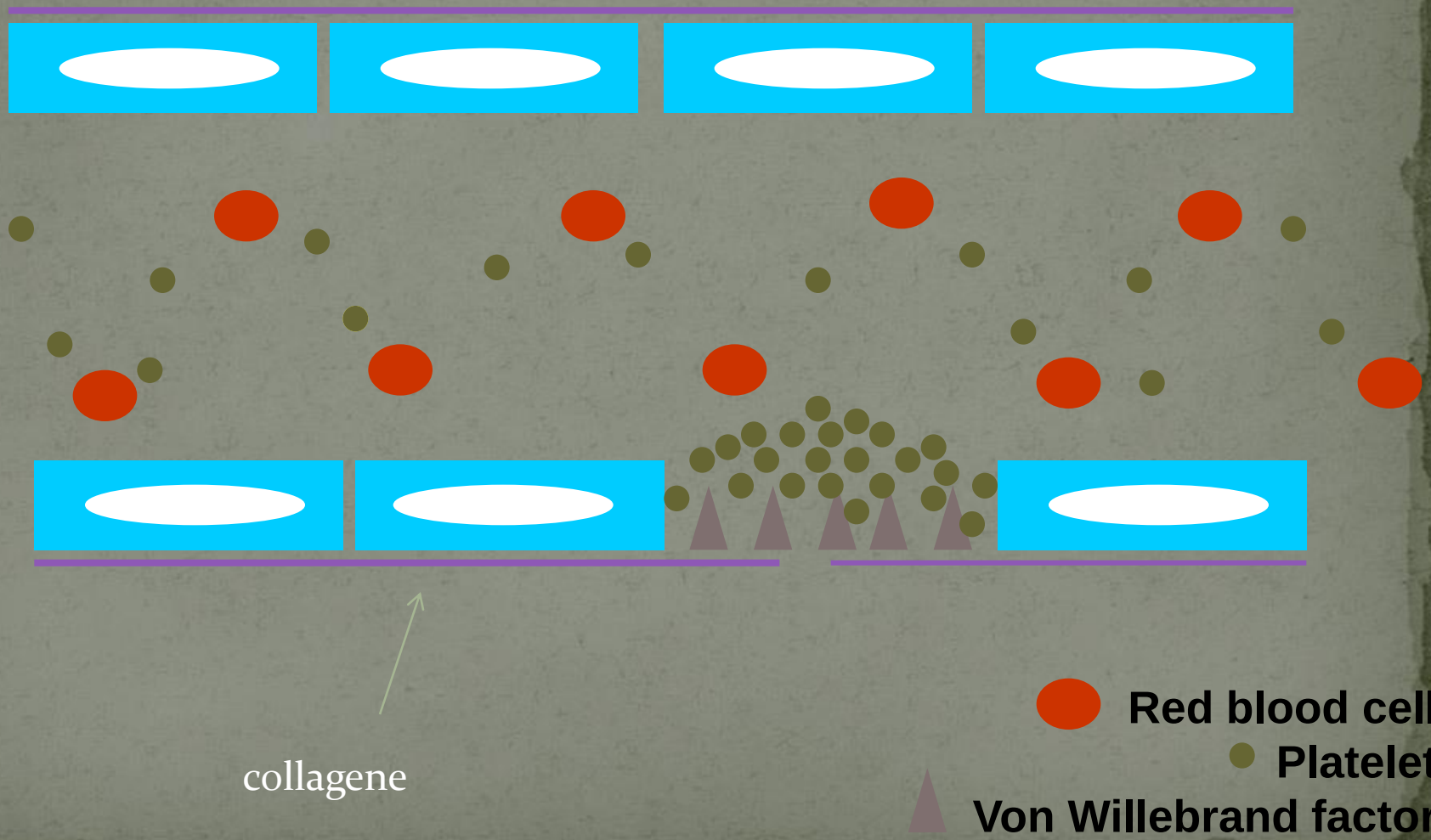

collagene
Red blood cell
Platelet
Von Willebrand factor

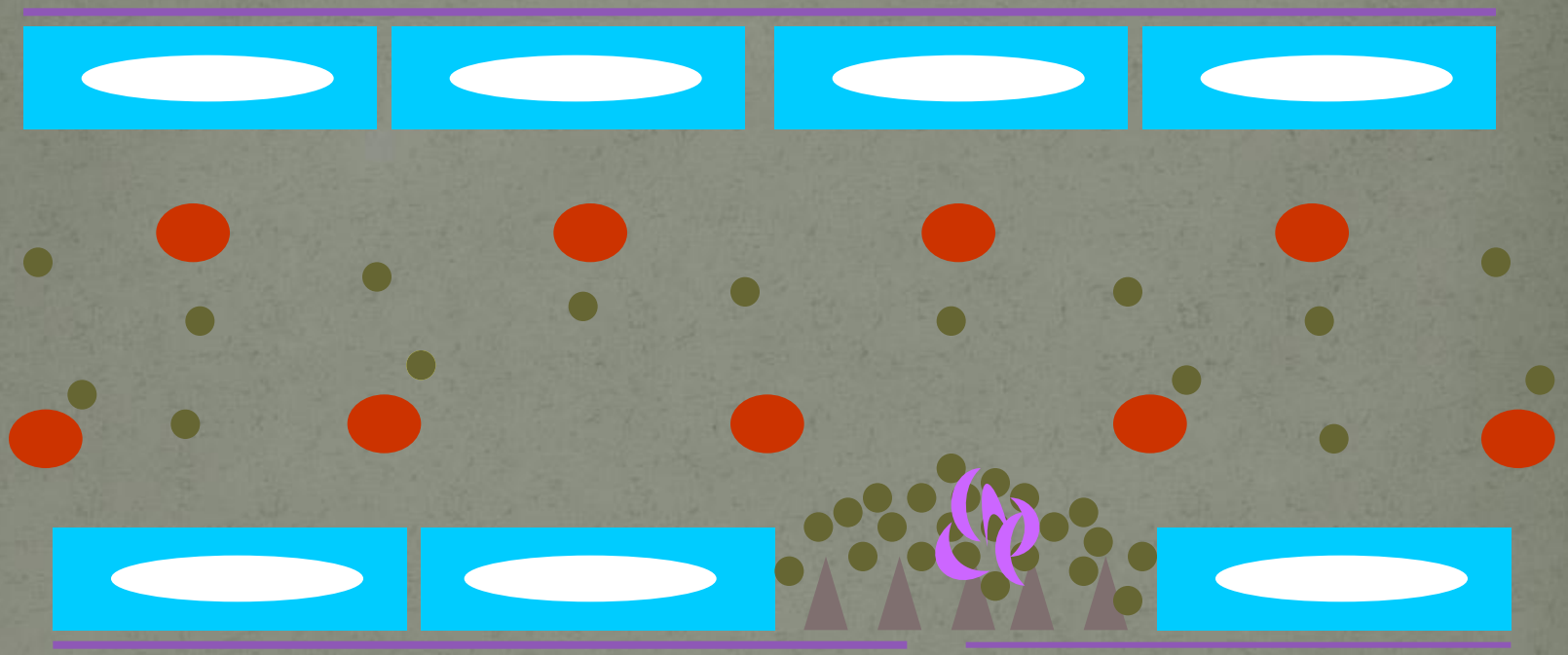

collagene
Red blood cell
Platelet
Von Willebrand factor

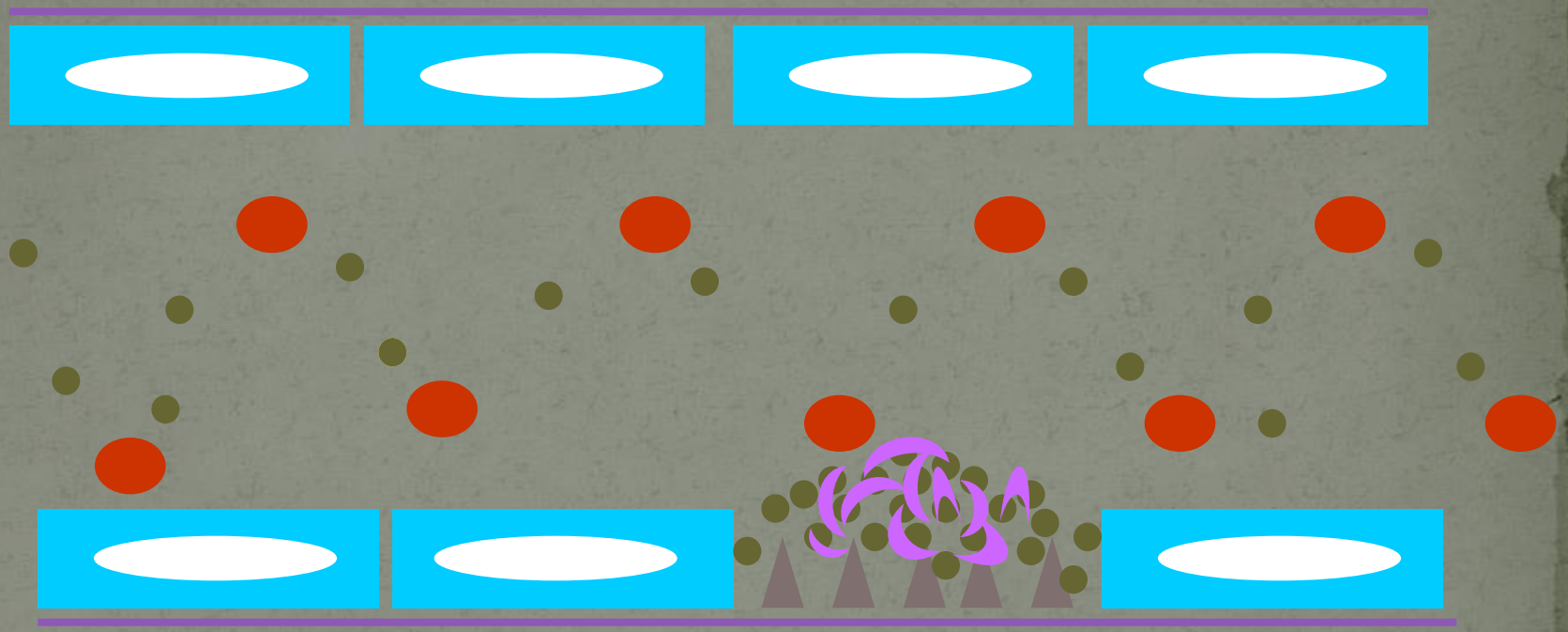

collagene
Red blood cell
Platelet
Von Willebrand factor

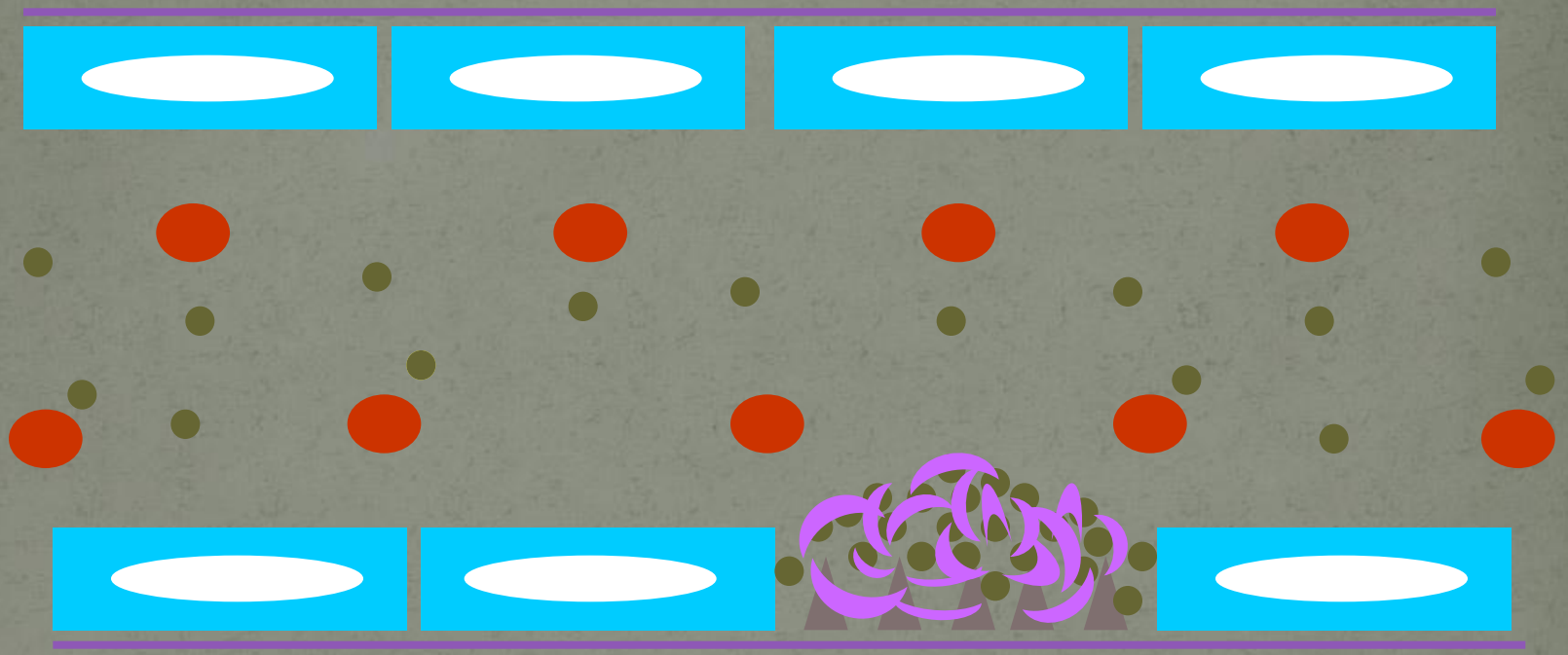

collagene
Red blood cell
Platelet
Von Willebrand factor


collagene
Red blood cell
Platelet
Von Willebrand factor

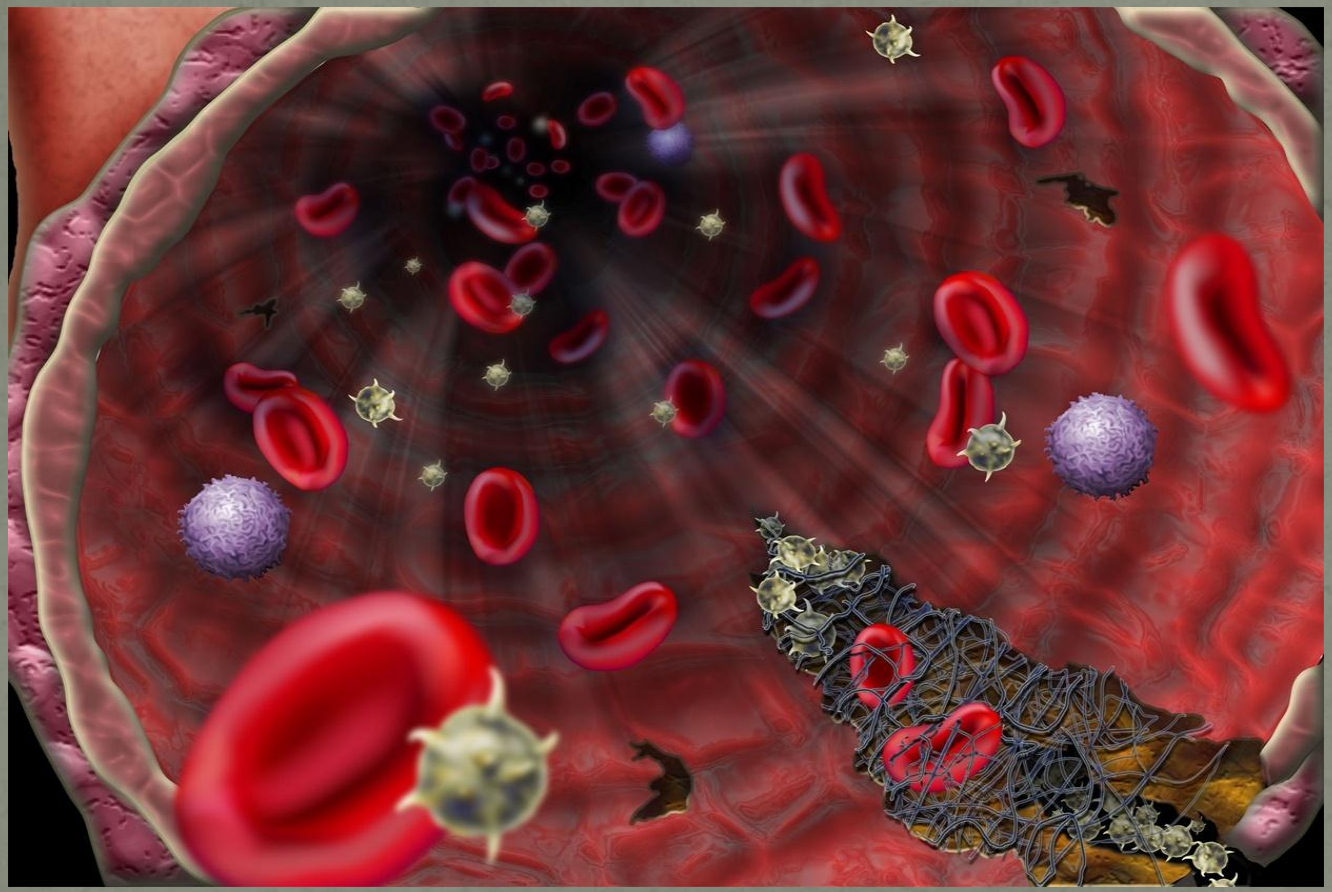

collagene
Red blood cell
Platelet
Von Willebrand factor


collagene
Red blood cell
Platelet
Von Willebrand factor
Fibrin polymer


collagene
Red blood cell
Platelet
Von Willebrand factor
Fibrin polymer


collagene
Red blood cell
Platelet
Von Willebrand factor
Fibrin polymer

Blood clot



Secondary Hemostasis

- During aggregation, phospholipid (PL) becomes available on the platelet membrane surface, providing a site for fibrin formation and thrombo-genesis (formation of blood clots).

- Secondary Hemostasis

  The Intrinsic and Extrinsic Coagulation Pathways

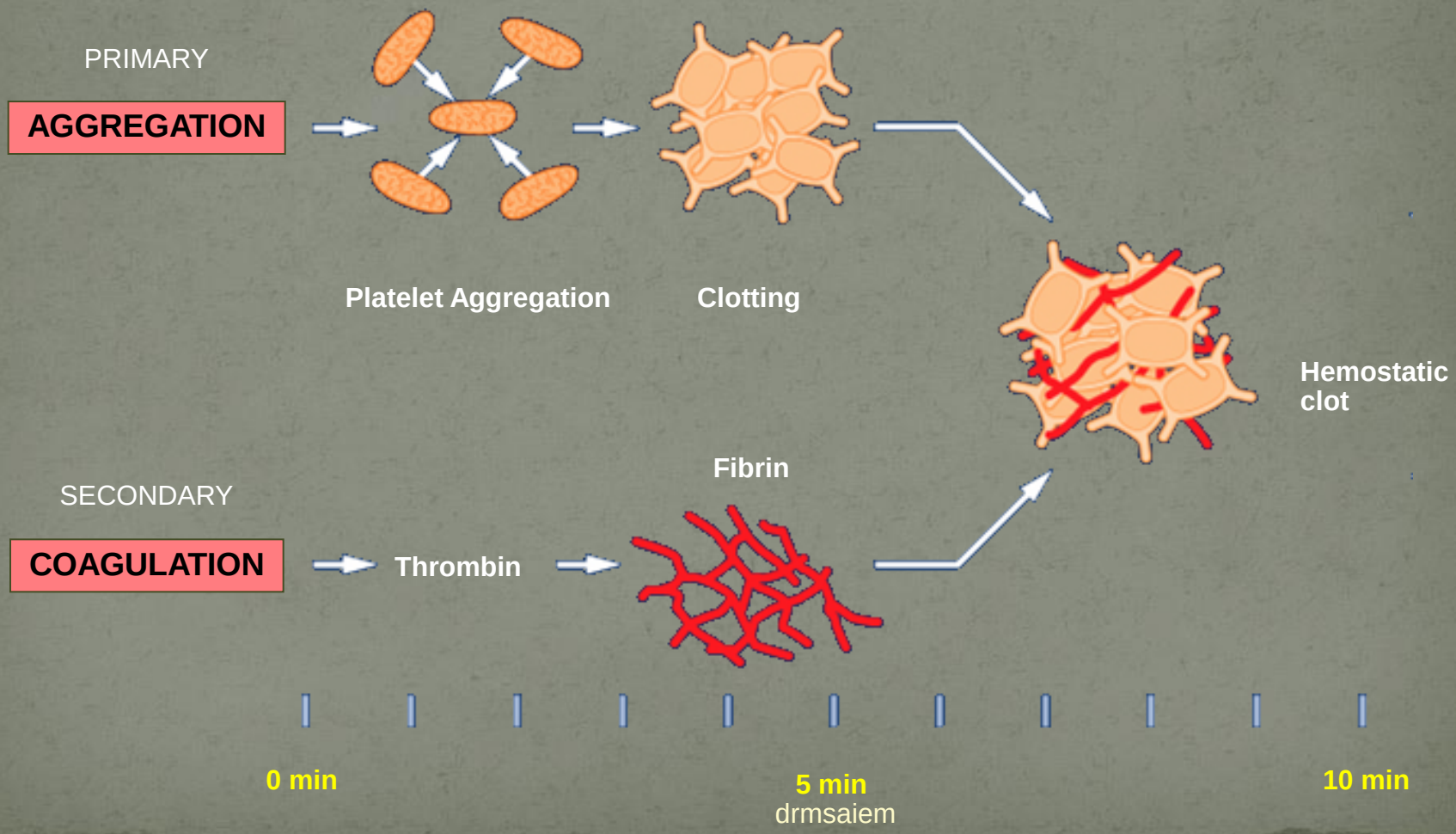
  The intrinsic system is activated in *vivo* by the contact of certain coagulation proteins with subendothelial connective tissue, which sets the secondary hemostatic mechanism into motion.

- The extrinsic coagulation pathway, in contrast, is initiated with the release of tissue factor from injured vessel endothelial cells and sub-endothelium into the vessel lumen.

- Tissue factor, a high-molecular-weight lipoprotein, is found in most organs, including the lungs, kidneys, liver, brain, placenta, and spleen, as well as in large blood vessels

- Both the intrinsic and the extrinsic coagulation pathways lead to secondary hemostasis, namely, the formation of the stable fibrin clot.
- The clot thus includes both fibrin formed in secondary hemostasis and the platelet plug formed in primary hemostasis.

Hemostatic Plug Formation



COAGULATION PROTEINS

- The intrinsic and extrinsic coagulation pathways are a series of reactions involve coagulation factors known as

  1- enzyme precursors (zymogens)

  2- non-enzymatic (cofactors)

  3- calcium (Ca^{++})

  4- phospholipids (PL).

- All coagulation factors normally are present in the plasma, with PL being provided by platelets.

- The zymogens are factors II, VII, IX, X, XI, XII, and prekallikrein
- The cofactors are factors V, VIII, tissue factor, and high-molecular-weight kininogen (HMWK).
- Zymogens are substrates that have NO biologic activity until converted by enzymes to active enzymes called serine proteases, which have exposed, serine-rich, active enzyme sites.
- Serine proteases selectively hydrolyzed arginine or lysine-containing peptide bonds of other zymogens, thus converting them to serine proteases.

COAGULATION GROUPS

- The properties of the coagulation and kinin factors have similarities that can divide these factors easily into three groups:

1- Contact group;

2- Prothrombin or vitamin K-dependent group;

3- Fibrinogen group.

- Contact Group

- Prekallikrein
- HMWK (high molecular Weight kallikrein) of the kinin group
- XII
- XI

The contact group is adsorbed by contact with a negatively charged surface such as collagen or the subendothelium in *vivo*

and glass *in vitro*.

- This contact causes slow conversion of factor XII to XIIa, which initiates both intrinsic system coagulation and fibrinolysis.

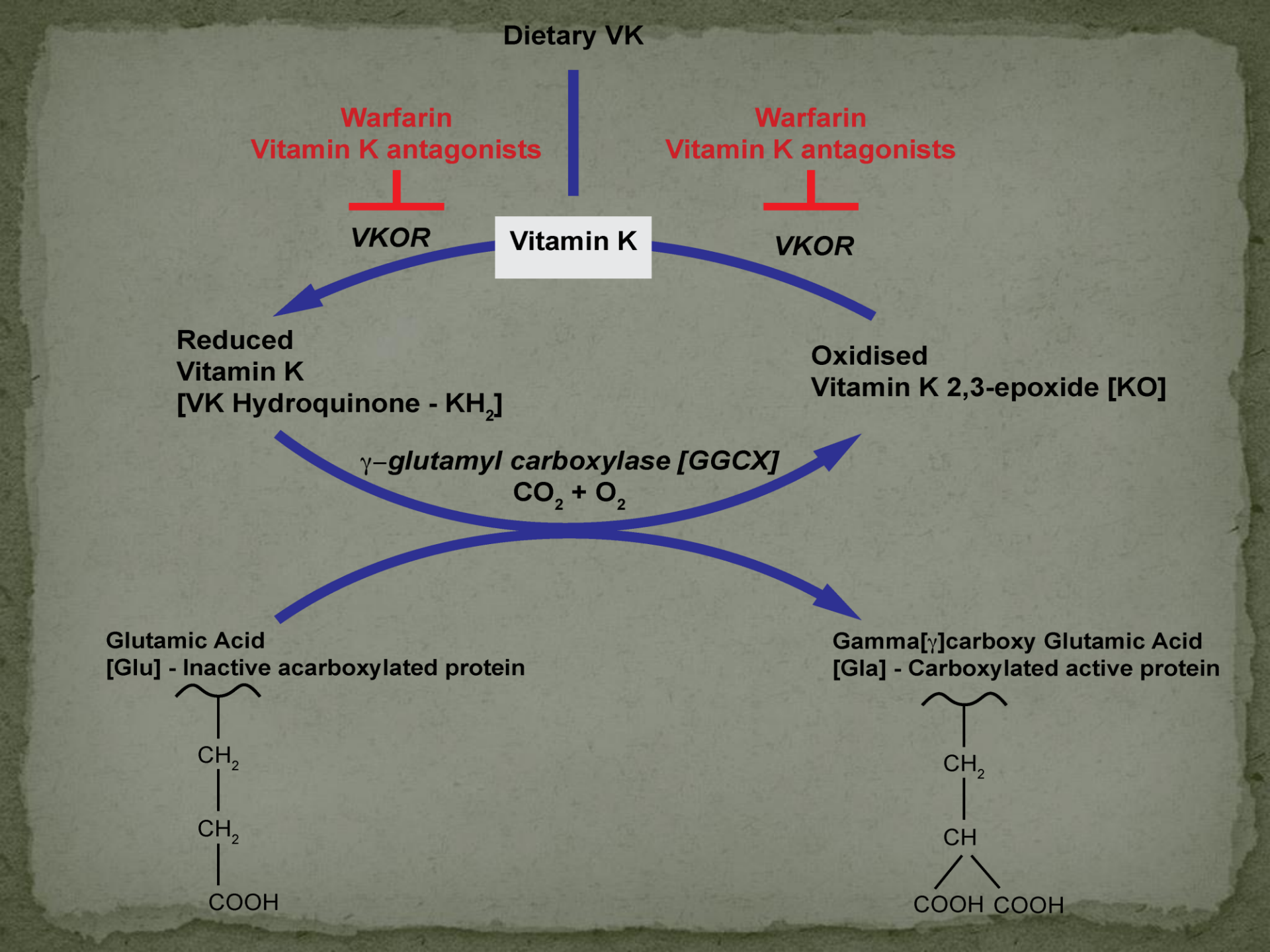
- Factor XIIa, and HMWK together activate factor XI to XIa, and convert prekallikrein to kallikrein.
- Kallikrein and HMWK together play a role in intrinsic coagulation activation, activation of fibrinolysis, kinin formation, and activation of the complement system.

Prothrombin (Vitamin K-Dependent) Group

- Contains the vitamin K-dependent coagulation factors

  II, VII, IX, and X.

- These factors are synthesized in the liver in the presence of vitamin K, which acts as a cofactor.
- Vitamin K is fat soluble. It is normally ingested in the diet and also is manufactured by the gut flora. There is no substantial storage of vitamin K in the body.
- Vitamin K is necessary to gamma-carboxylate the pre-formed enzyme precursors of factors (II, VII, IX, and X)


Dietary VK
Warfarin
Vitamin K antagonists
Warfarin
Vitamin K antagonists
VKOR
Vitamin K
VKOR
Reduced
Vitamin K
[VK Hydroquinone - KH2]
Oxidised
Vitamin K 2,3-epoxide [KO]
γ–glutamyl carboxylase [GGCX]
CO2 + O2
Glutamic Acid
[Glu] - Inactive acarboxylated protein
CH2
CH2
COOH
Gamma[γ]carboxy Glutamic Acid
[Gla] - Carboxylated active protein
CH2
CH
COOH COOH

- Many mechanisms can cause the formation of non-functional vitamin K-dependent coagulation factors.
- When such factors are released to the circulation, they cannot bind to the platelet PL surface and ultimately prevent Prothrombin activation, causing a deficiency in the coagulation pathway.

Fibrinogen Group

The fibrinogen group includes

- fibrinogen (factor I)
- V
- VIII
- XIII.

These have:

the highest molecular weights of all factors

are the most labile

are consumed in coagulation,

are the only group that act as substrates for the fibrinolytic enzyme plasmin.

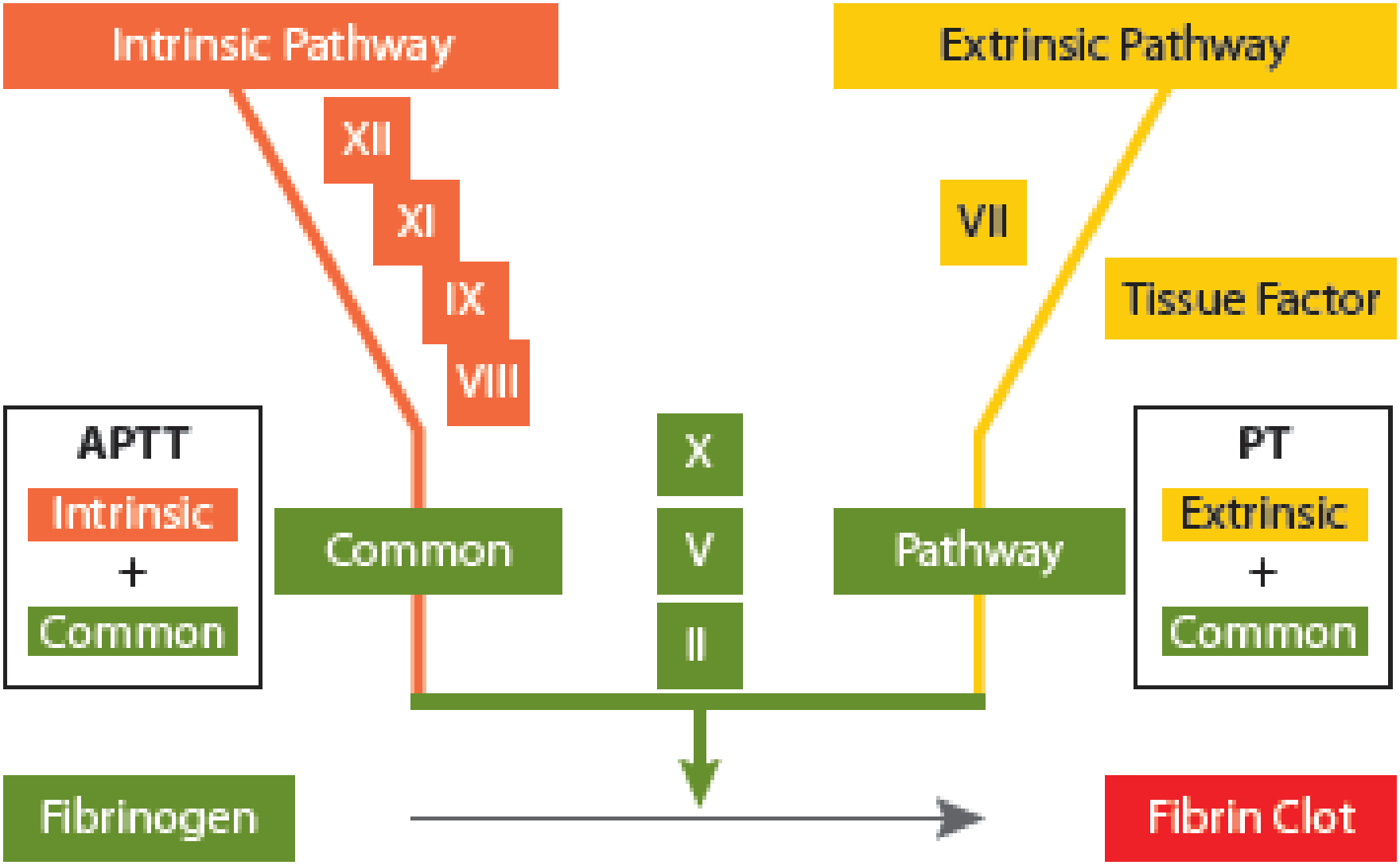
- Only the factors found in the fibrinogen group are found in the platelets, specifically in the alpha granules with two exceptions:
- (1) factor XIII is found in the general platelet cytoplasm not in alpha granules
- (2) factor VIII:C, the coagulant portion of factor VIII, is not found in platelets.

PHOSPHOLIPIDS CONTRIBUTING TO COAGULATION

- Tissue Factor
- The existence of a lipoprotein called Thromboplastin (a complex of two parts, a PL and a protein).
- This substance initiates the extrinsic coagulation pathway by binding its PL portion to factor VII, converting factor VII to VIIa.

- The term extrinsic was applied to this pathway because of the necessity of adding a tissue extract (PL) to plasma samples in *vitro* to initiate and evaluate this coagulation pathway in the laboratory.

- The Prothrombin Time (PT) test which evaluates the extrinsic system, is performed using a reagent contained (rabbit brain) or lung tissue Thromboplastin as well as Ca^{++} to activate factor VII and initiate the extrinsic pathway.


Intrinsic Pathway
Extrinsic Pathway
XII
XI
IX
VIII
VII
Tissue Factor
APTT
Intrinsic
+
Common
Common
X
V
II
Pathway
PT
Extrinsic
+
Common
Fibrinogen
Fibrin Clot

NATURAL INHIBITORS OF THE COAGULATION CASCADE

Role: to limit clotting to the area where it is needed

Blood flow and hepatic degradation of clotting factors:

- Normal blood flow dilutes the activated clotting factors below the level required to propagate the cascade.

- Hepatocytes in the liver digest and destroy the activated clotting factors washed away from the site of clot formation.

Antithrombin III:

- is the most important physiologic inhibitor of activated coagulation factors.
- synthesized in the liver and endothelial cells.
- irreversibly binds to and inhibits thrombin, factor Xa, and other activated clotting factors.
- Heparin (or heparan sulfate on endothelial cells) binds to and activates AT.
- By itself,AT has a low affinity for thrombin; however, complexing with heparin increases the activity of AT approximately 2,000-fold.

Protein C and protein S:

- are vitamin K–dependent
- inhibitors of the coagulation cascade that control coagulation by inactivating factors Va and VIIIa.
- **Protein C** is activated by the binding of thrombin to thrombomodulin on endothelial cell surfaces;
- therefore, ***thrombin***, *a key mediator of the coagulation cascade, also initiates a key anticoagulant system*

Protein C and protein S: cont.

- When thrombin binds to thrombomodulin, it enzymatically cleaves and activates protein C.
- Activated protein C (APC), in combination with protein S, inactivates factors Va and VIIIa.
- Protein S circulates in two forms: free protein S and protein S complexed with a protein involved in the complement system, the C4b binding protein.
- Free protein S is active, whereas the bound form is not.

FIBRINOLYTIC SYSTEM

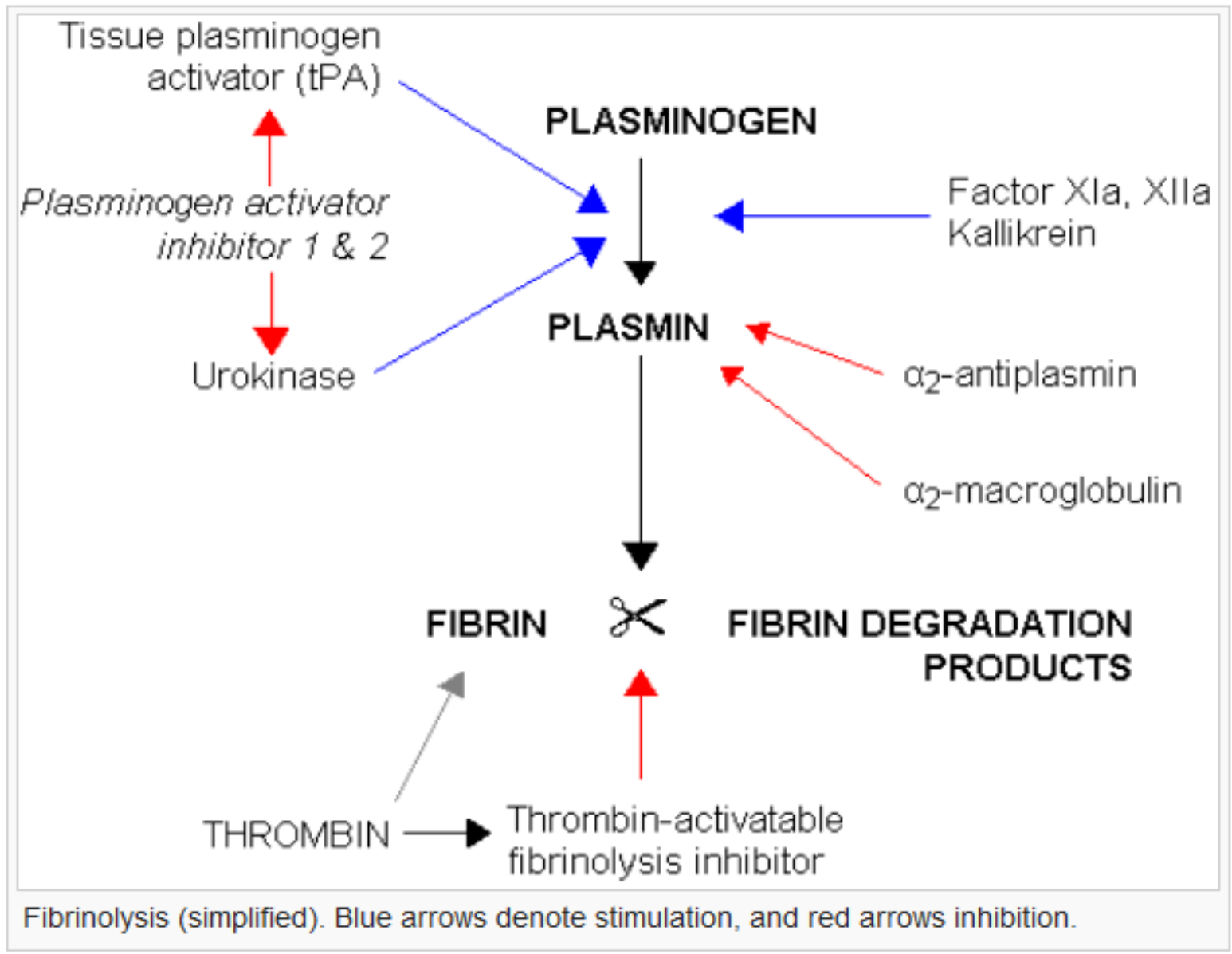
The important players in fibrinolysis are:

- plasminogen/plasmin
- t-PA (tissue- Plasminogen Activator).

- inhibitors of plasminogen activation:

- ✓ Alpha 2-antiplasmin
- ✓ PAI s (plasminogen activator inhibitor)

Fibrinolysis.



Fibrinolysis (simplified). Blue arrows denote stimulation, and red arrows inhibition.

Plasminogen/Plasmin

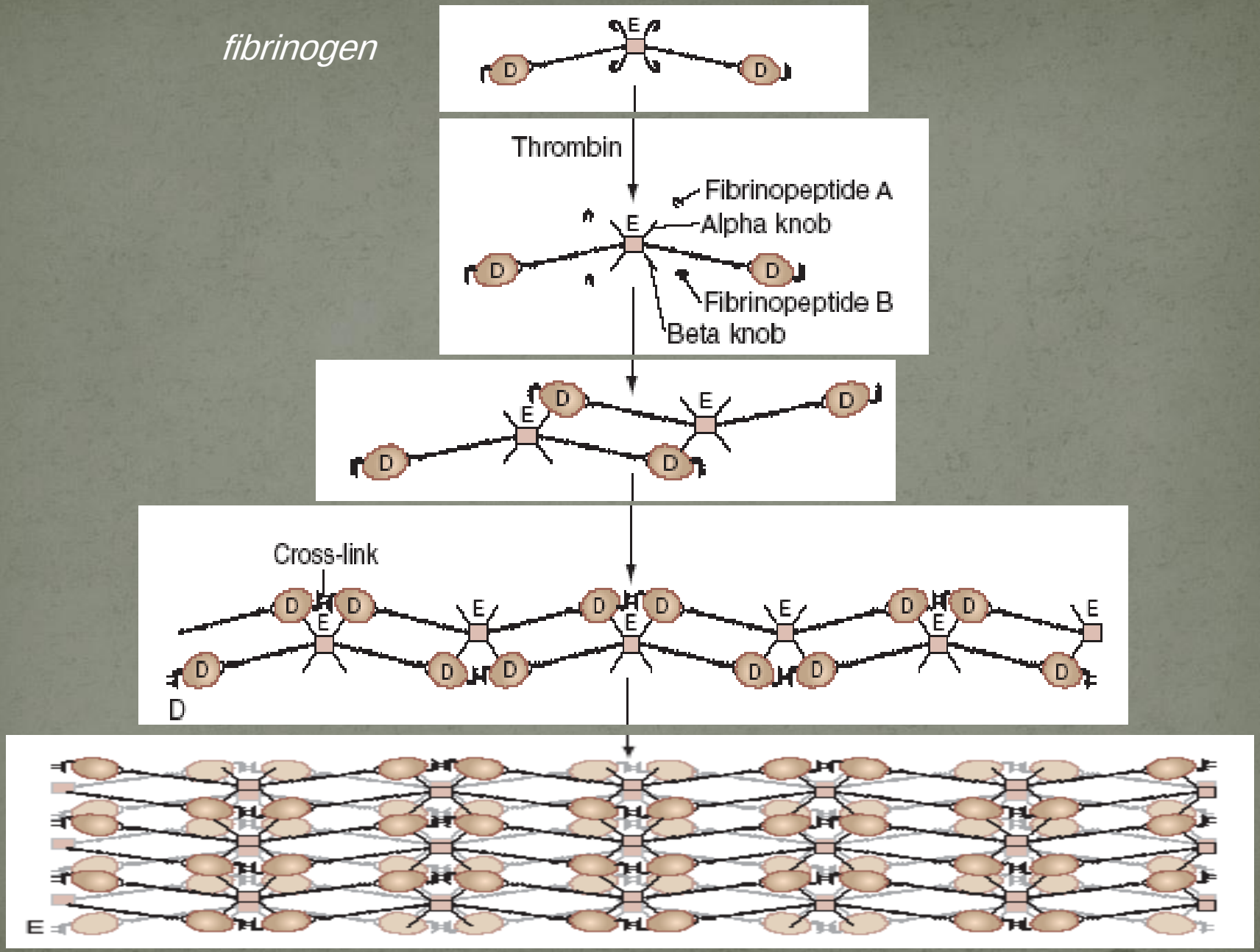
- *Plasmin is the enzyme that digests fibrin and thus dissolves clots.*

Plasminogen is activated to plasmin by

- t-PA,which is secreted by endothelial cells
- the contact factor (XII, HMWK, and PK). This appears to be a **minor** activator in vivo.

- **The results of fibrin degradation by plasmin are:**
- **FDP** (fibrin degradation products)
- **D-Dimer**

fibrinogen

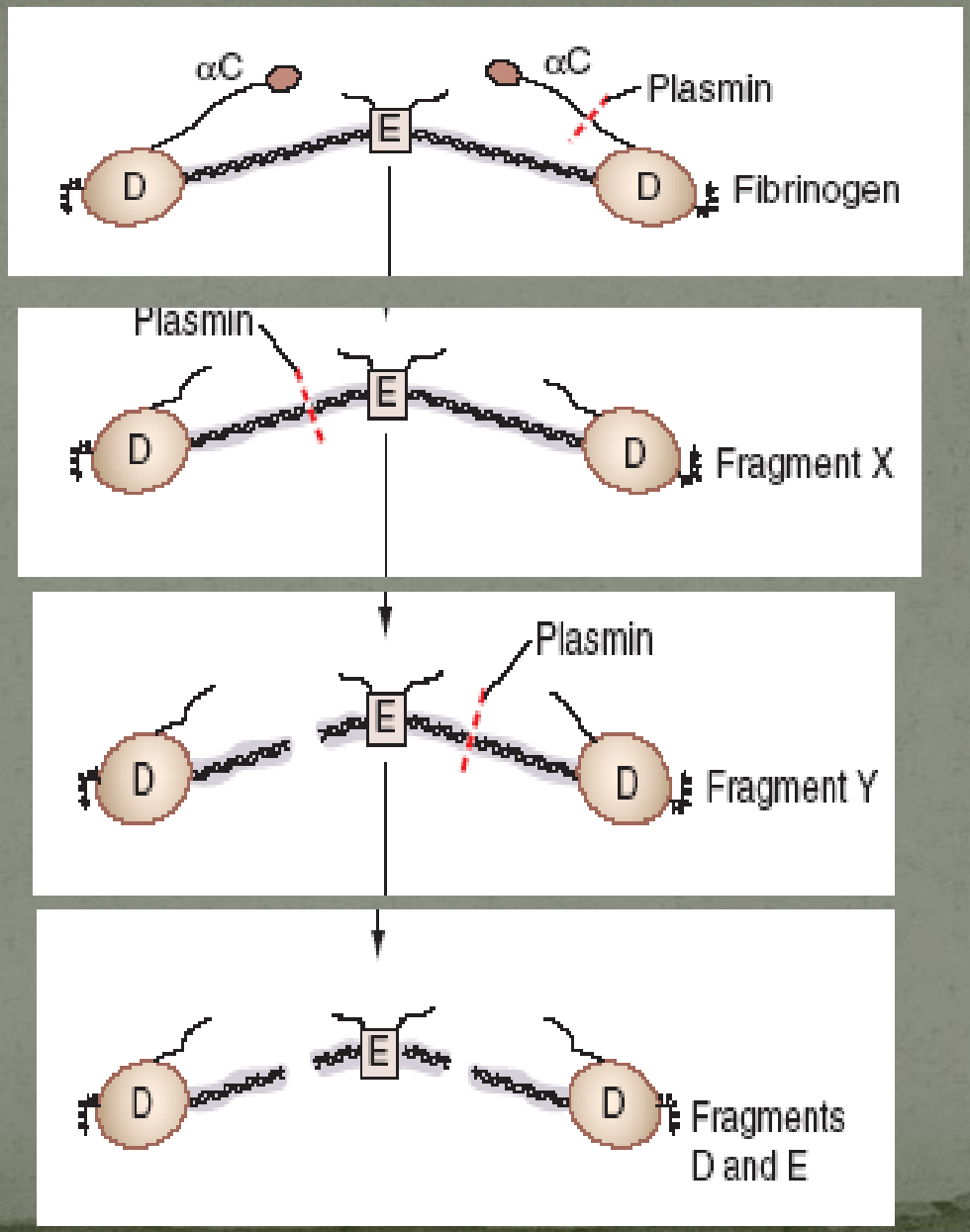

E
D
D
Thrombin
Fibrinopeptide A
Alpha knob
E
D
D
Fibrinopeptide B
Beta knob
D
E
E
D
D
D
Cross-link
D
E
D
D
E
D
D
D
E
D
D
E
D
D
E
D
D
E
D
D
D
D
E

FDP

- Plasmin can digest fibrinogen in thrombotic events
- plasmin can also digest fibrinogen in non-thrombotic events (structural defect in fibrinigem) and result in a positive test for FDPs

- inhibit coagulation by inserting into the fibrin clot in place of fibrinogen
- They also inhibit platelet aggregation.

FDPs are not actually specific for *fibrin degradation;*


αC
αC
Plasmin
E
D
D
Fibrinogen
Plasmin
E
D
D
Fragment X
Plasmin
E
D
D
Fragment Y
E
D
D
Fragments
D and E

FDP

D-Dimer

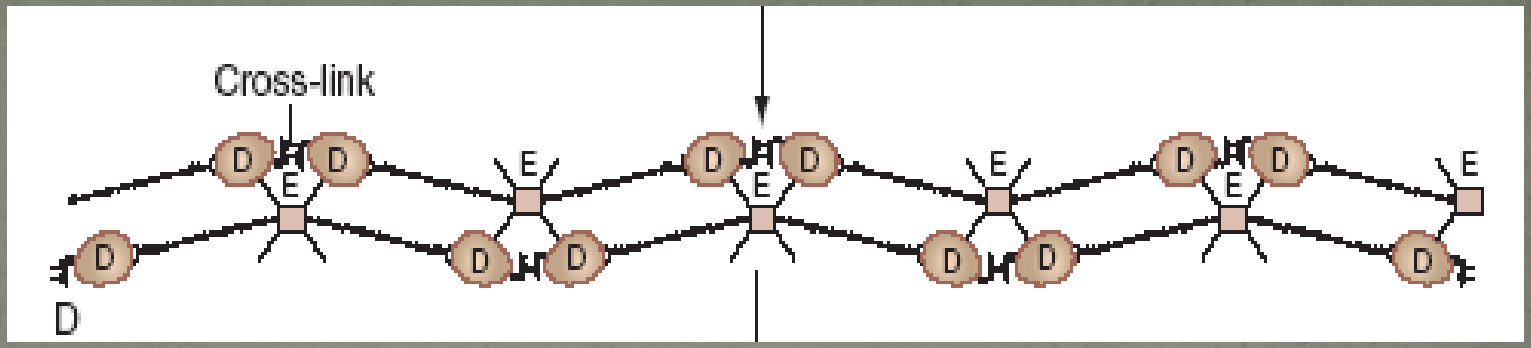
A negative test for D-dimer is evidence against a significant thrombus

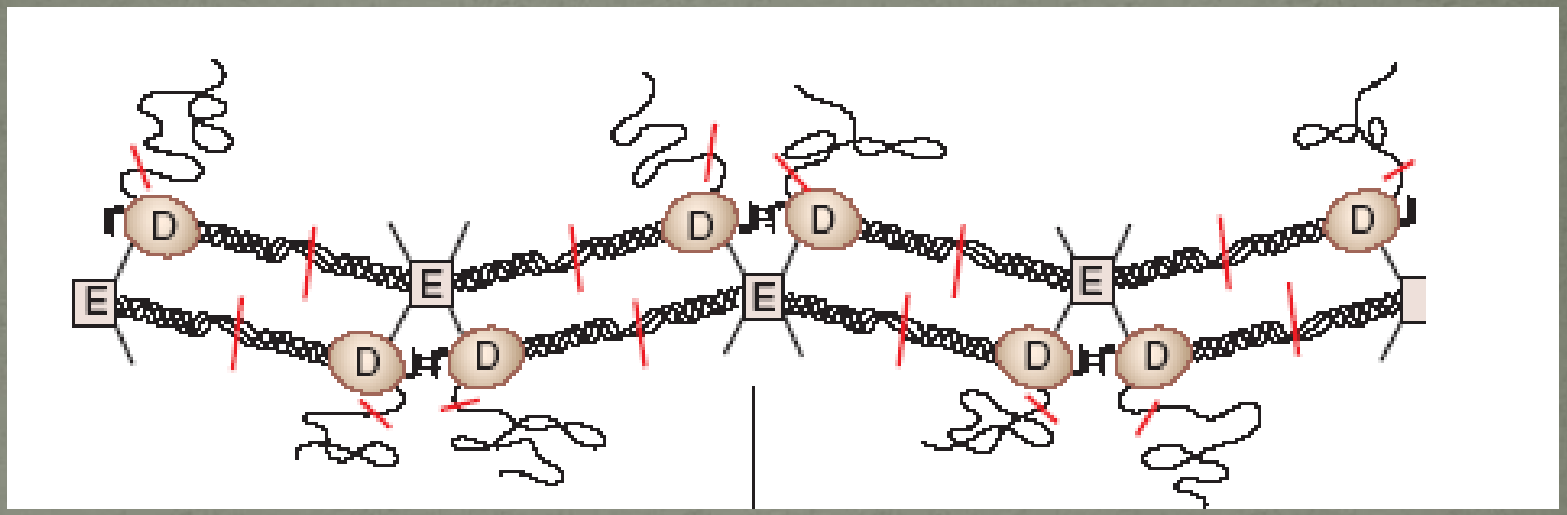
- A specific fibrin degradation product
- results from the digestion of fibrin that has been crosslinked
- by factor XIIIa.
- **Thus, the presence of D-dimer in circulation indicates that:**

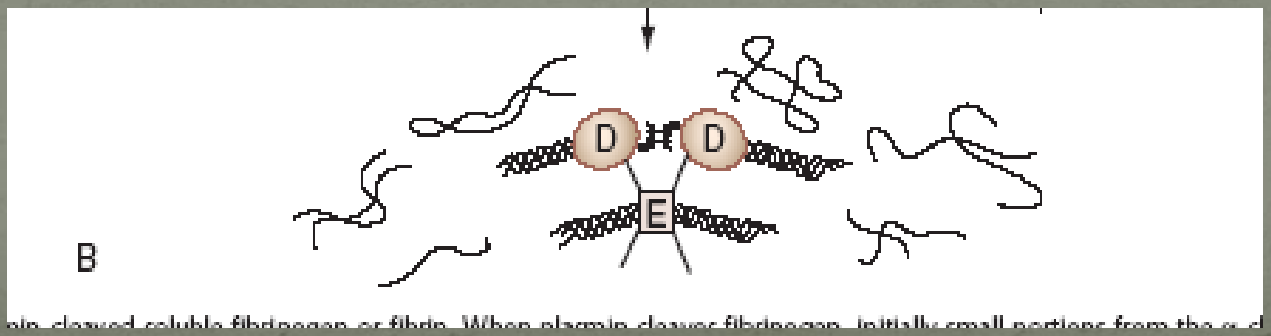
✓ thrombin has been activated and has resulted in both **fibrin clotting**

✓ activation of factor XIII to XIIIa,

✓ plasminogen has been activated to plasmin with subsequent digestion of the cross-linked fibrin clot.







D-Dimer

Control of fibrinolytic system

- excessive activity of the fibrinolytic system can result in severe bleeding
- One important control mechanism is **localization of plasmin activity to the surface of fibrin clots**.
- **t.PA**
  - ✓ has a much higher affinity for plasminogen that is localized on the surface of a fibrin clot than it does for free plasminogen,
  - ✓ this helps to specifically localize fibrinolyis to the clot.
- **Alpha 2-antiplasmin**
  - ➢ which inactivates any plasmin that is free in circulation
  - ➢ Plasmin bound to fibrin is protected from inhibition by it
- **PAI**
  - ❖ inhibitors of plasmin activation

Disorders of Hemostasis

- **Vascular disorders –**
  - Scurvy, easy bruising, Henoch-Schonlein purpura.
- **Platelet disorders**
  - **Quantitative** - Thrombocytopenia
  - **Qualitative** - Platelet function disorders – Glanzmans
- **Coagulation disorders**
  - **Congenital** - Haemophilia (A, B), Von-Willebrands
  - **Acquired** - Vitamin-K deficiency, Liver disease
- **Mixed/Consumption:** DIC

Disoders of Primary hemostasis

QUANTITATIVE PLATELET DISORDERS

- Thrombocytopenia

<100,000/μl	BT prolonged
≈10,000	Bleeding in trauma or OR
<10,000	Spontaneous, CNS bleeding

- Thrombocytopenia due to destruction
  - ITP (acute in children, chronic in young women) with anti-glycoprotein
  - Drug reaction
  - Heparin induced thrombocytopenia
  - DIC and TTP

ABOUT THROMBOTIC THROMBOCYTOPENEIC PURPURA (TTP)

- Disorder of systemic platelet aggregation in microvasculature
- Stimulus: unusually large vWf
- In children: likely to be deficiency in vWf metalloproteinase to break down vWf
- In adults: vWf metalloproteinase inhibited by autoantibodies
- Low PLT count, intravascular hemolysis, RBC fragmentation, high LDH

IDIOPATHIC THROMBOCYTOPENIC PURPURA (ITP)

- Caused by an autoreactive antibody to the patient's platlets
  - Young children – acute and usually transient for 1-2 weeks with spontaneous remission
  - Adults – chronic and occurs more often in women
  - Treatment
    - Corticosteroids
    - Splenectomy
    - Rituximab

QUANTITATIVE PLATELET DISORDERS

- Thrombocytopenia due to decreased production
  - Aplastic anemia (e.g., Fanconi's)
  - Fibrosis
  - Acute leukemia
  - Megaloblastic anemia
  - Hereditary (e.g., May-Hegglin, Wiscott-Aldrich, Bernard-Soulier)
- Splenic sequestration
- HELLP syndrome (hemolysis, elevated liver enzyme, low PLT) in pre-eclampsia
- Dilution (massive transfusion)

Disoders of secondary hemostasis

Disorders of plasma clotting factors

Disorders of Secondary Hemostasis

- Disorders of the proteins of fibrin formation
- Disorders of proteins associated with fibrinolysis
- Symptoms of secondary hemostatic disorders
  - Delayed bleeding
  - Deep muscular bleeding
  - Spontaneous joint bleeding
  - Symptoms common to primary and secondary hemostatic disorders
    - Ecchymoses
    - GI bleeding
    - Hematuria
    - Hypermenorrhea
    - Gingival bleeding
    - Increased bleeding after tooth extraction
    - Intracranial bleeding
    - Epistaxis

Disorders of Proteins of Fibrin Formation

- Hereditary vs acquired
- Quantitative vs qualitative deficiencies
  - Laboratory screening tests (PT, APTT)
    - Does not differentiate quantitative vs qualitative disorders
  - Qualitative abnormal proteins will
    - Prolong clotting test
    - Be recognized by immunologically-based procedures
- Activity assays
  - Essential when screening for deficiencies

von Willebrand Disease

- Inherited hemorrhagic disorder
  - Genetically and clinically heterogeneous
  - Caused by a deficiency/dysfunction of VWF
  - Most common hereditary bleeding disorder
- VWF
  - Multimeric blood protein
  - Performs two major roles in hemostasis
    - Mediates adhesion of platelets to sites of vascular injury
    - Is a carrier protein for F-VIII
- Inherited defects in VWF may
  - Interfere with biosynthetic processing or disrupt specific ligand binding sites
  - Cause bleeding by impairing either platelet adhesion or blood clotting
  -

VWD

- Three major categories of VWD
  - Type 1 VWD – partial quantitative deficiency of VWF
  - Type 2 VWD – qualitative deficiency of VWF
    - Divided into 4 variants
    - Type 2A – ↓ platelet-dependent function
      - Absence of high-molecular weight VWF multimers
    - Type 2B – ↑ affinity for platlet GPIb
    - Type 2M – ↓ platelet-dependent function
      - Not caused by the absence of HMW multimers
    - Type 2N – Markedly ↓ affinity for F-VIII
  - Type 3 VWD – total deficiency of VWF
  - Types 1 and 2 – autosomal dominant inheritance
  - Type 3 – autosomal recessive inheritance
- Diagnosis
  - Specific tests
    - Quantify VWF and F-VIII activity

VWD – Clinical Manifestations

- Hemorrhagic tendency is highly variable
  - Depends on the type and severity of disease
  - Patients with Type 1 and 2 disease
    - May have mild bleeding symptoms
    - Characterized by hemorrhage from delicate mucocutaneous tissues
    - Epistaxis, easy bruising, GI bleeding, menorrhagia
    - Hematoma and hemarthroses, characteristic of hemophilia A, are not prominent
  - Patients with severe type 3 VWD
    - Severe hemorrhagic tendency
    - Spontaneous hemorrhage
      - Mucous membranes, GI tract
      - Can be frequent and may be life threatening
    - Low F-VIII level
      - Deep hematomas
      - Joint hemorrhages – similar to hemophilia

Hemophilias

- Hemophilia A
  - Factor VIII Deficiency
    - Antihemophilic Factor
    - X-linked recessive disorder
    - Most common type of hemophilia
- Hemophilia B
  - Factor IX Deficiency
    - Christmas Factor (from family of first patients diagnosed with the disorder)
    - X-linked recessive disorder
- Hemophilia C
  - Factor XI Deficiency
  - Autosomal recessive disorder seen primarily in the Ashkenazi Jewish population
  - Symptoms range from mild to severe

Disseminated Intravascular Coagulation

- Normal balance of hemostasis is altered
- Results in the uncontrolled inappropriate formation and lysis of fibrin within the blood vessels
- Activation of coagulation occurs systemically
  - Rather than locally at site of injury
- Fibrin is deposited diffusely within capillaries, arterioles and venules
- Clotting proteins, inhibitors and platelets are consumed faster than they are synthesized
  - Acquired deficiency of multiple hemostatic components
  - Fibrinolysis follows fibrin formation
  - Patient generally bleeds spontaneously at the same time that disseminated clotting is occurring

DIC – Laboratory Diagnosis

- Laboratory diagnosis is difficult
  - Available tests are nonspecific
  - No single test can establish the definitive diagnosis of DIC
  - PT, APTT, TT prolonged
  - Fibrin degradation products are (+)
  - Platelet count ↓; platelet function tests abnormal
  - Schistocytes, thrombocytopenia on peripheral blood smear