بسم الله الرحمن الرحیم

هست کلید در گنج حکیم

مروری کوتاه بر سیستم هموستاز
و تست های مرتبط

Hemostasis = Love !!!!!!!

Every body talk about it

But

just a few people understand it !!!!!!!

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 = uncontrolled hemorrhaging or clotting

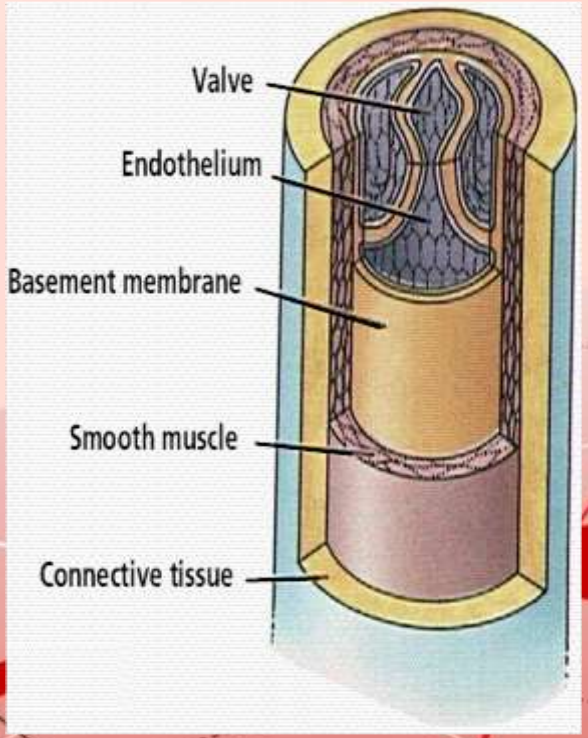
Following injury, each component must function optimally.

Haemostasis 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



- Minimizes blood flow to injured area
- Prevents blood loss
- Immediate
- Short-lived

Platelet

stages of platelet activation and plug formation

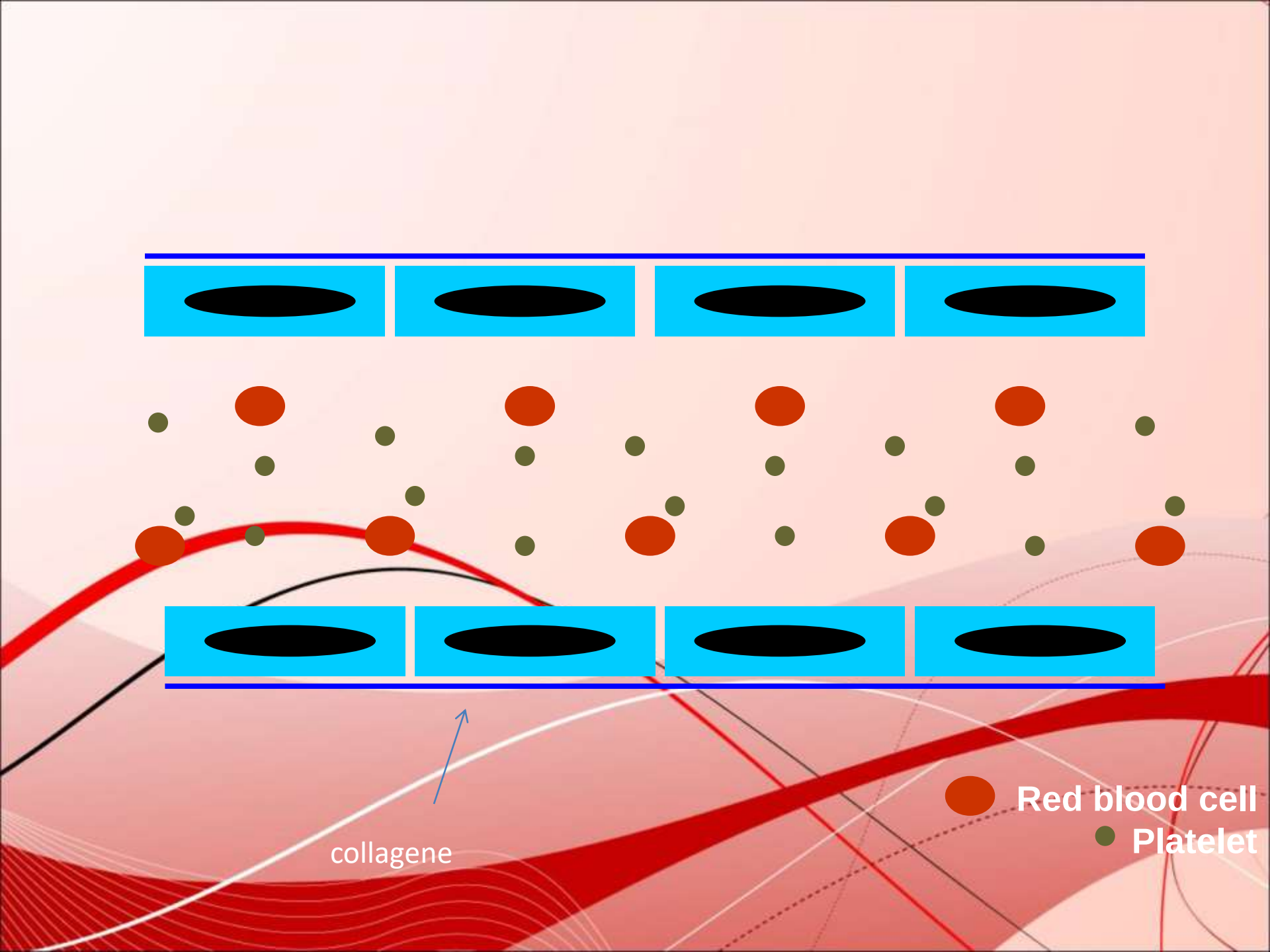
Stage 1: Platelet Adhesion

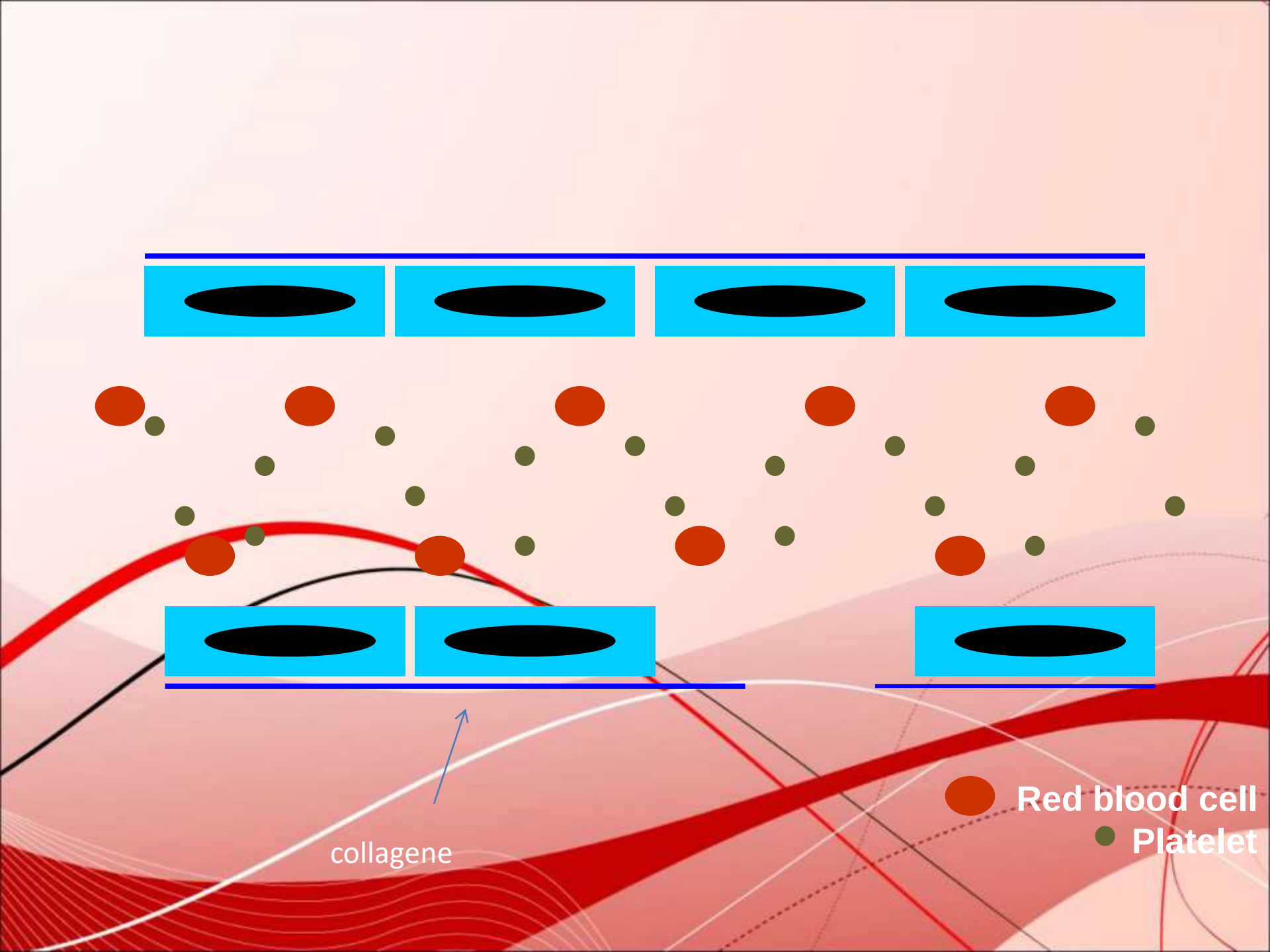
Stage 2: Platelet Aggregation

Stage 3: Platelet Secretion

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 "PF3" in this context)


collagene
Red blood cell
Platelet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Red blood cell
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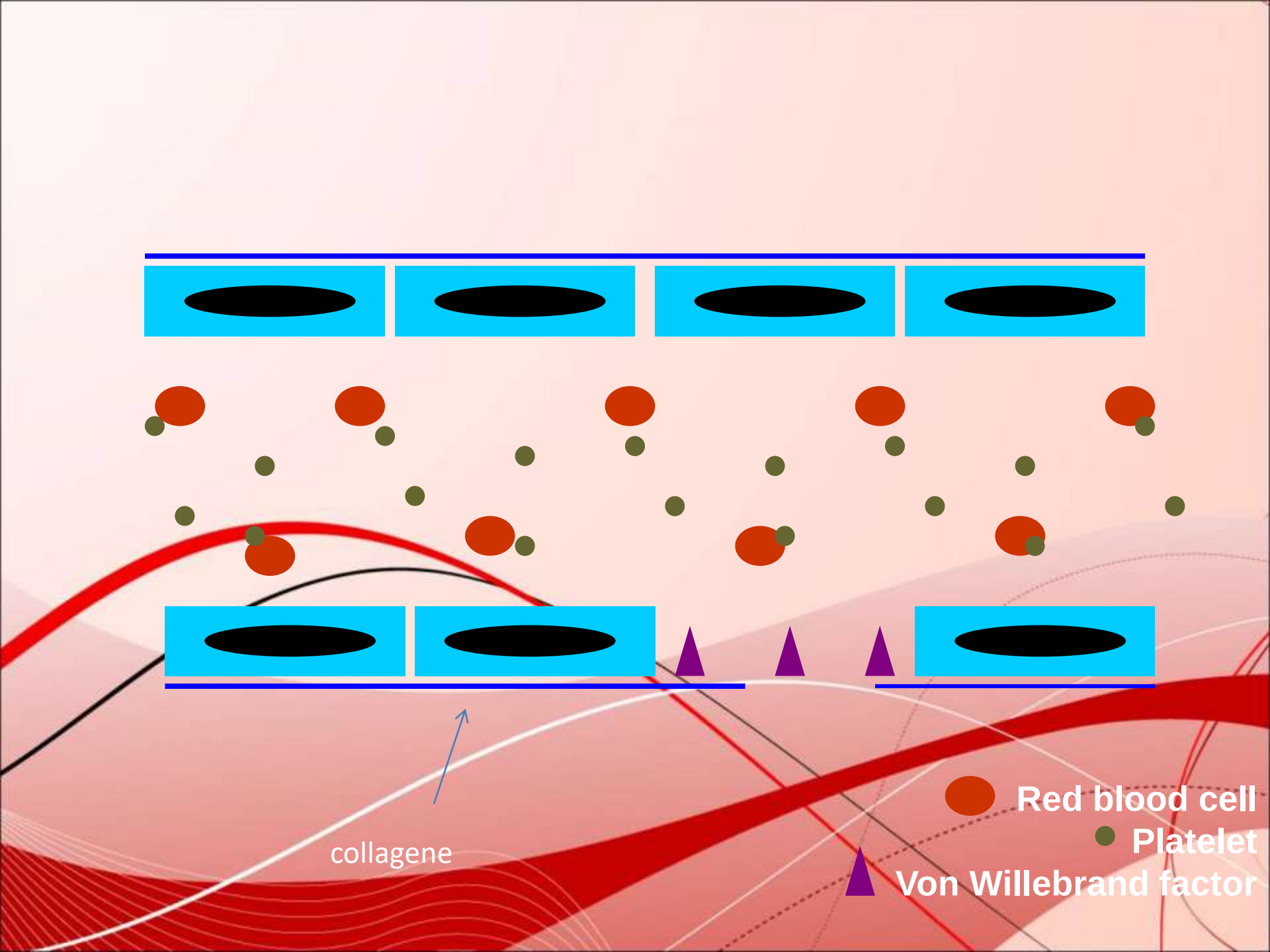
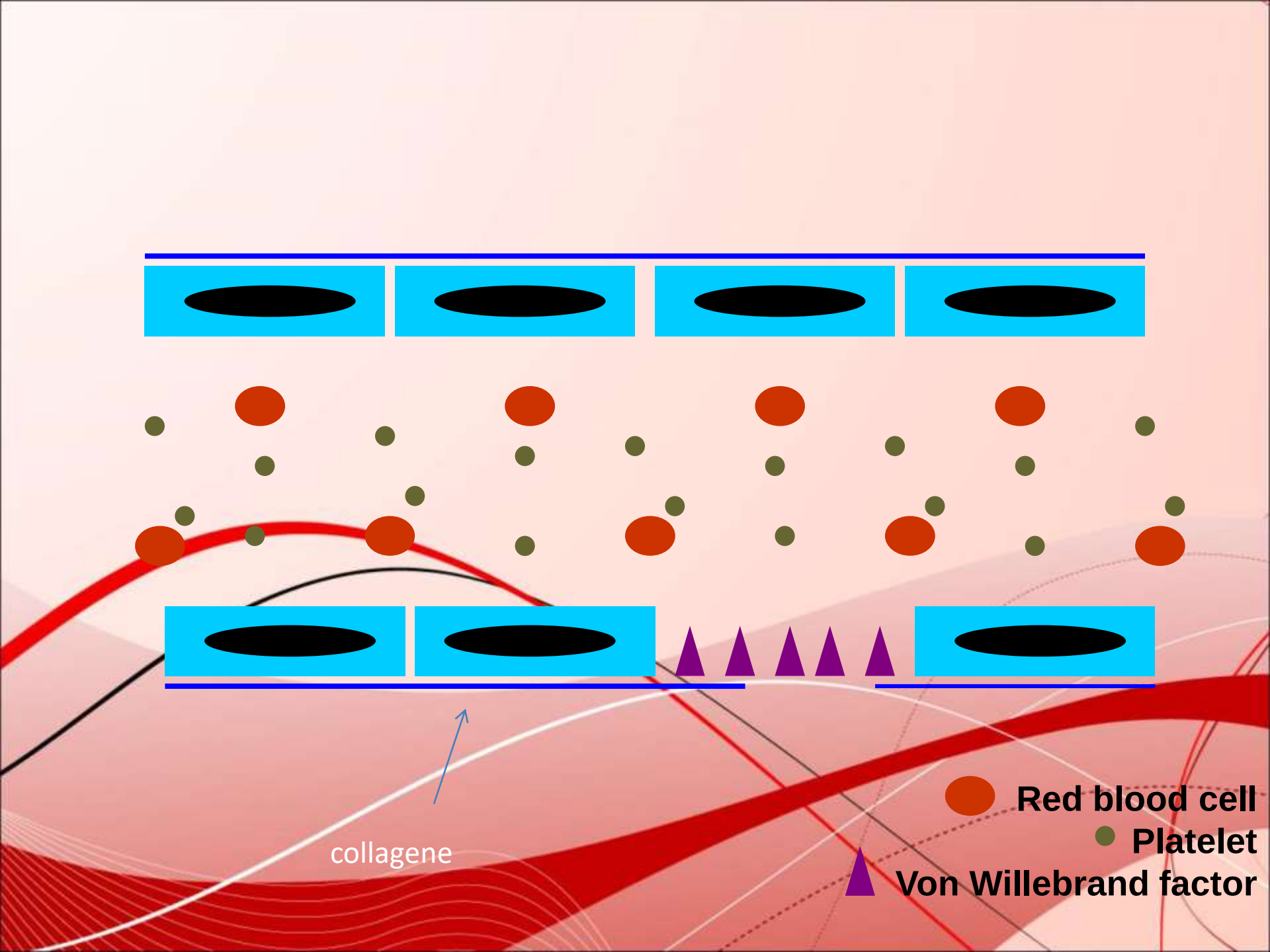

collagene
Red blood cell
Platelet
Von Willebrand factor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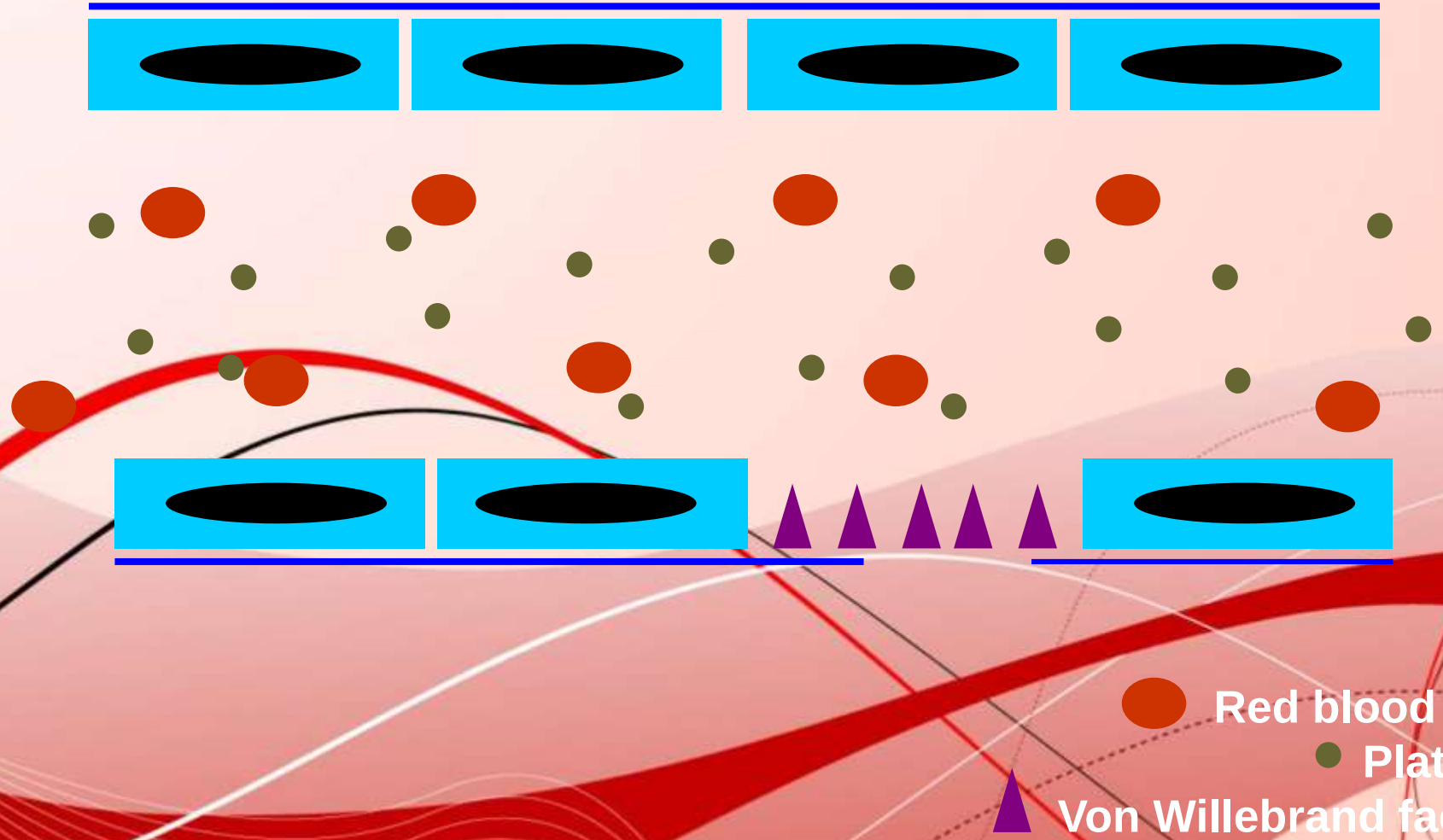
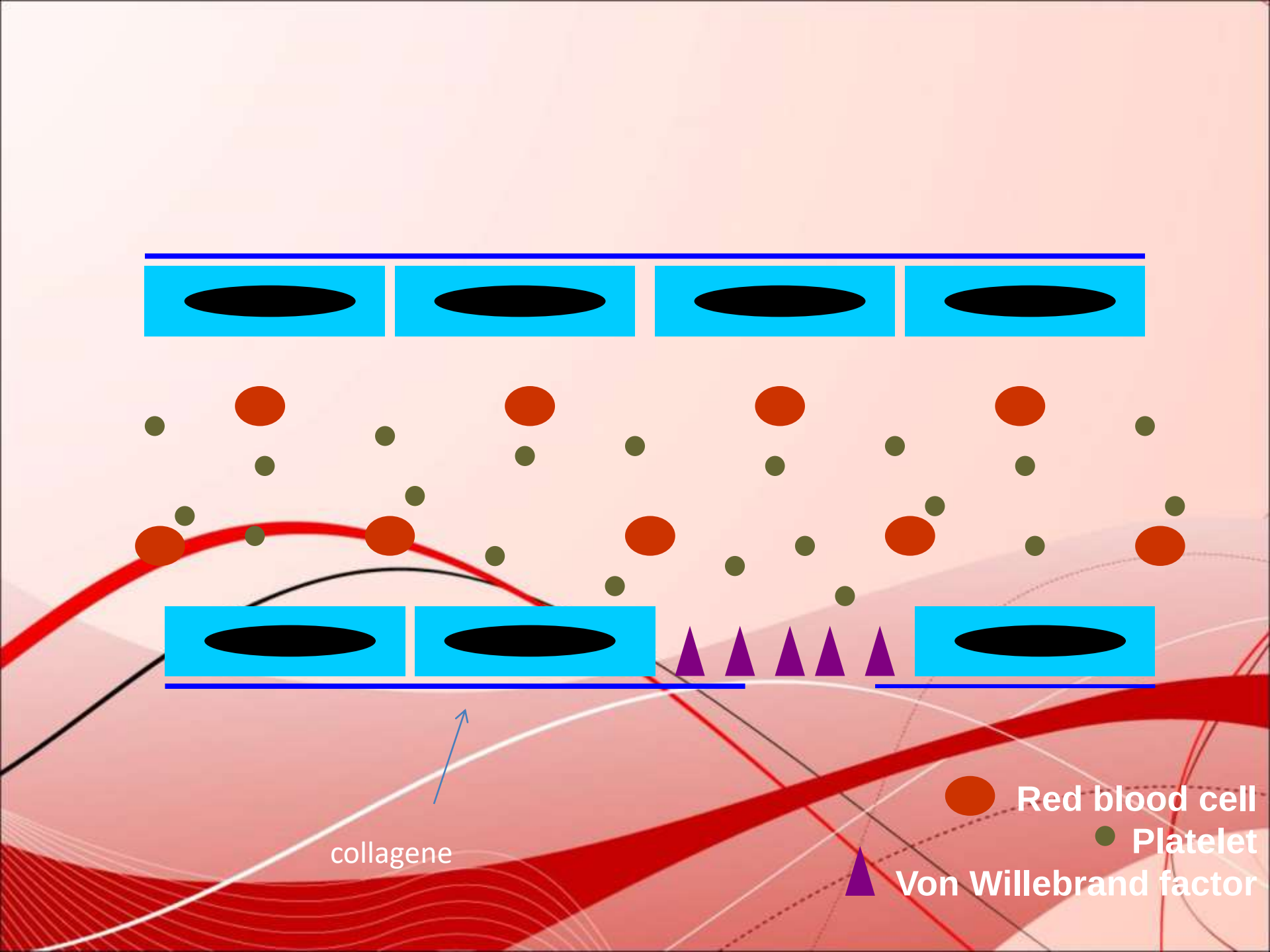
Red blood cell
Platelet
Von Willebrand factor


Red blood cell
Platelet
Von Willebrand factor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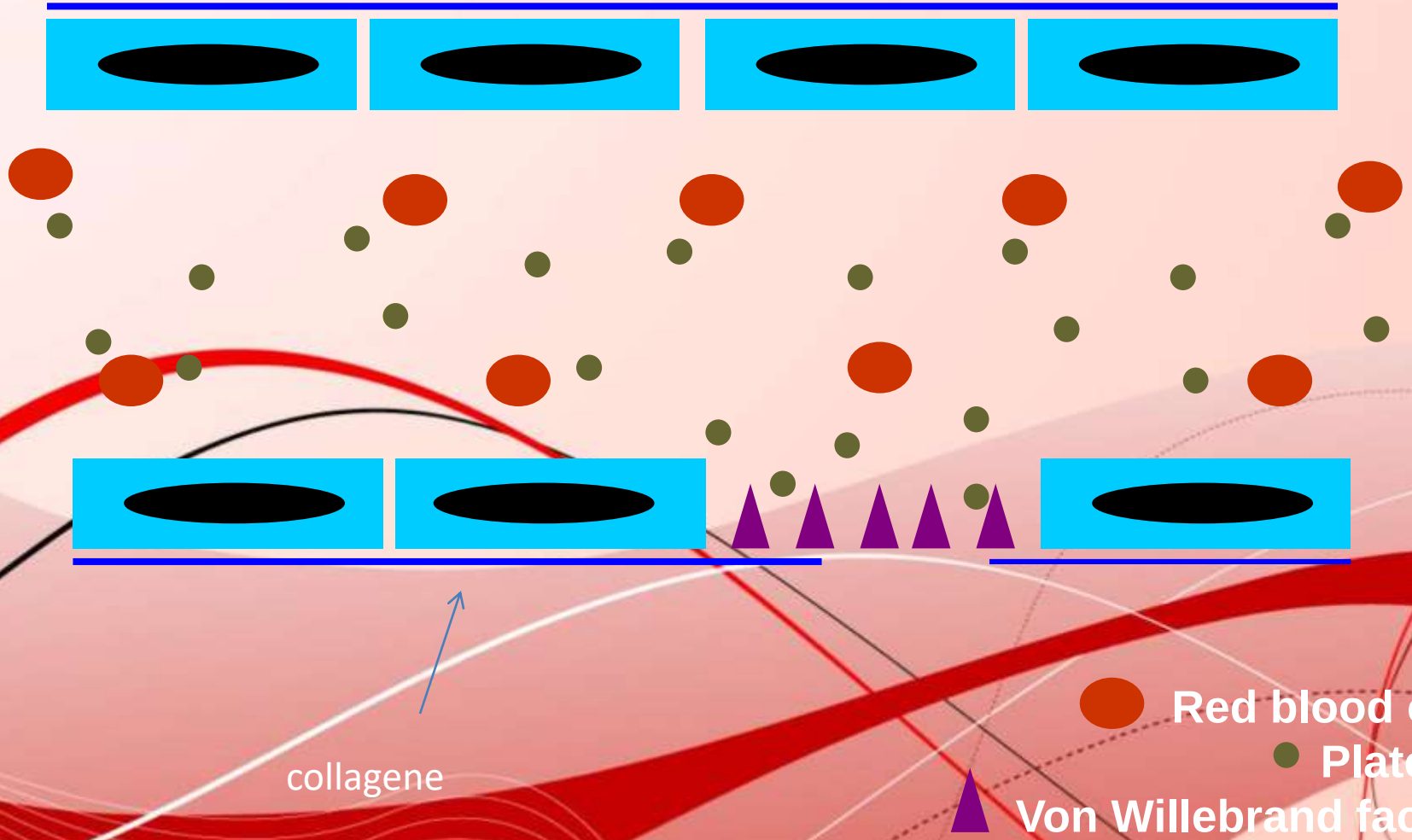
Platelet
Von Willebrand factor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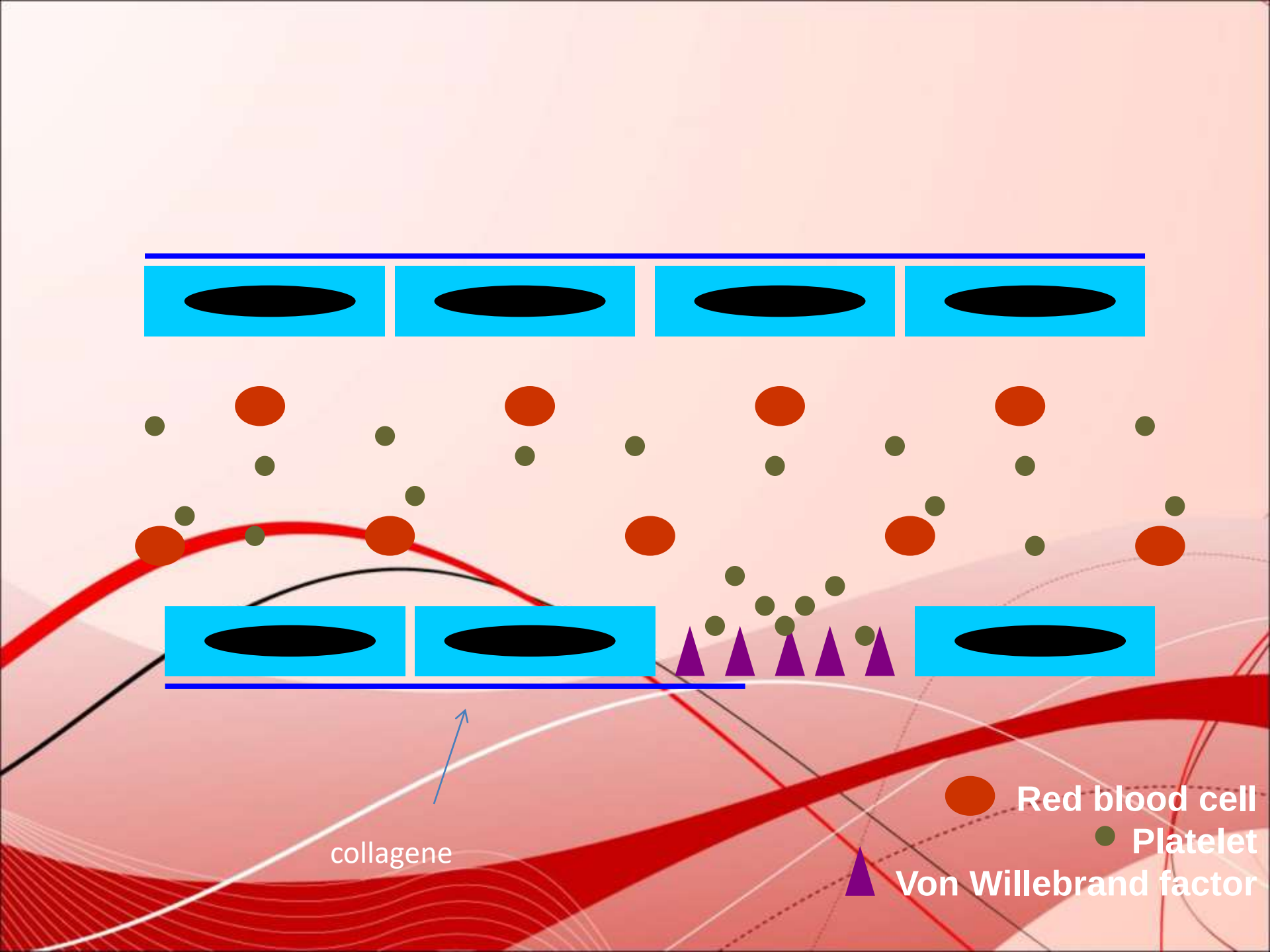
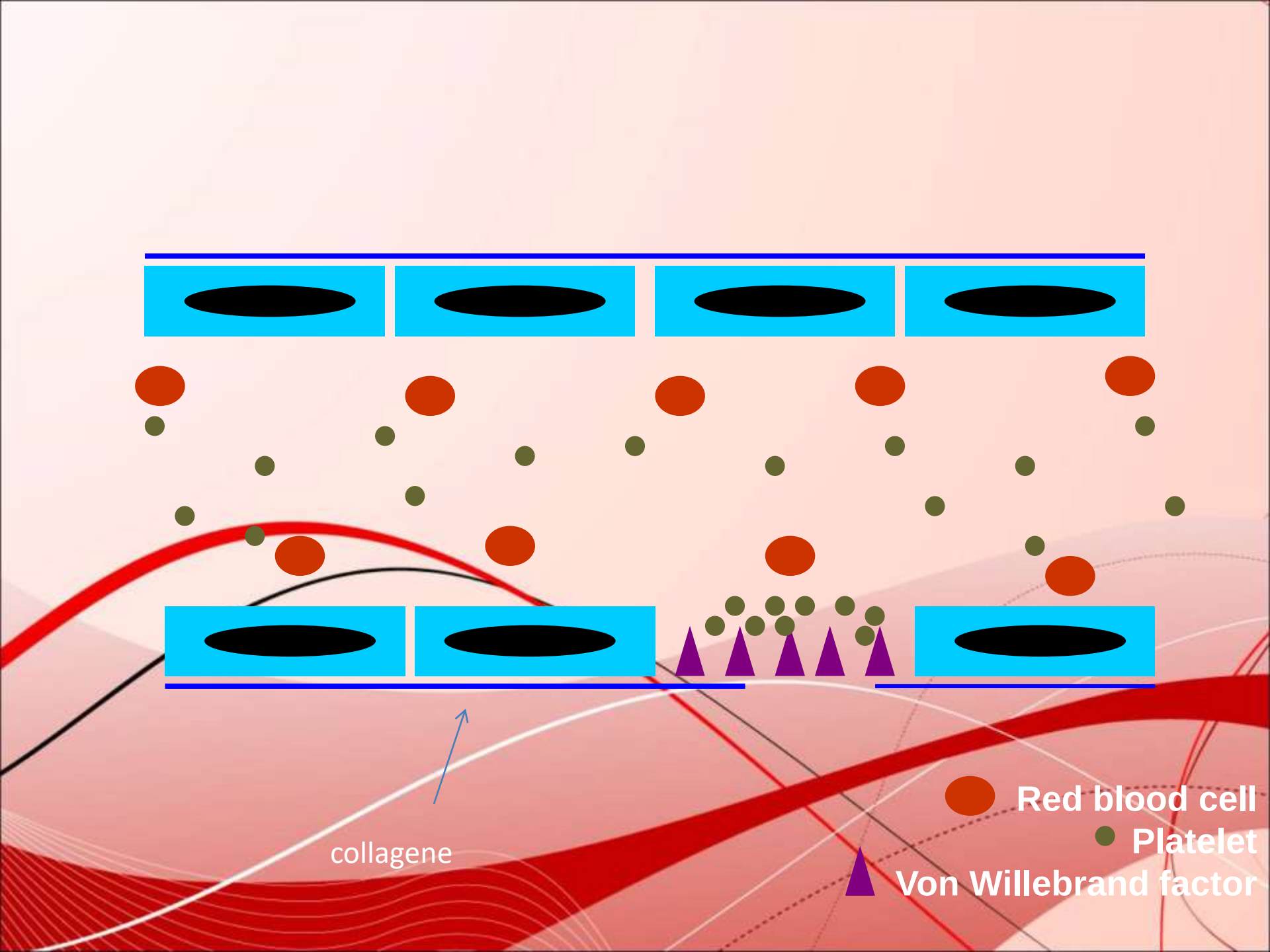
Red blood cell
Platelet
Von Willebrand factor


collagene
Red blood cell
Platelet
Von Willebrand factor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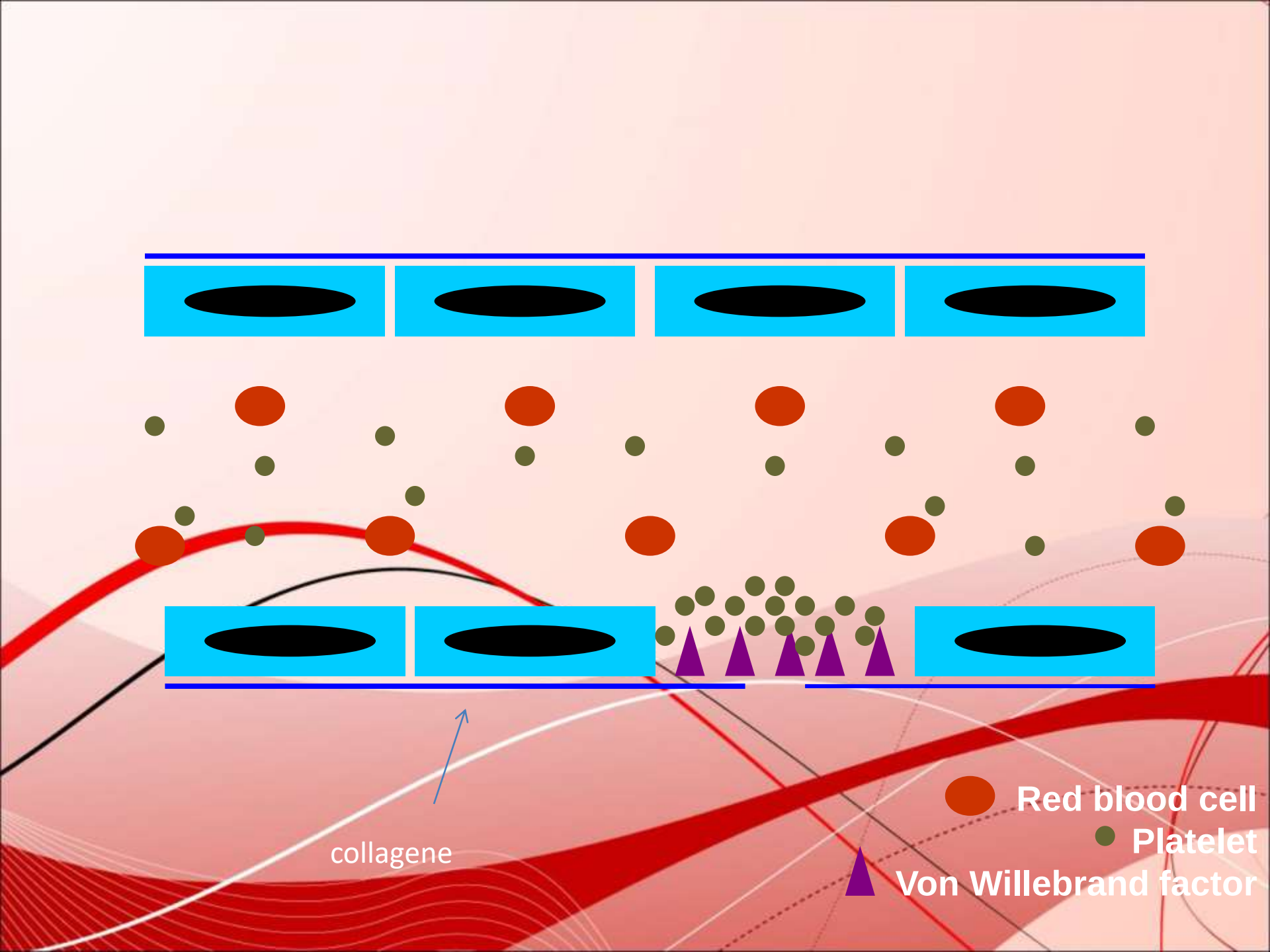
Red blood cell
Platelet
Von Willebrand factor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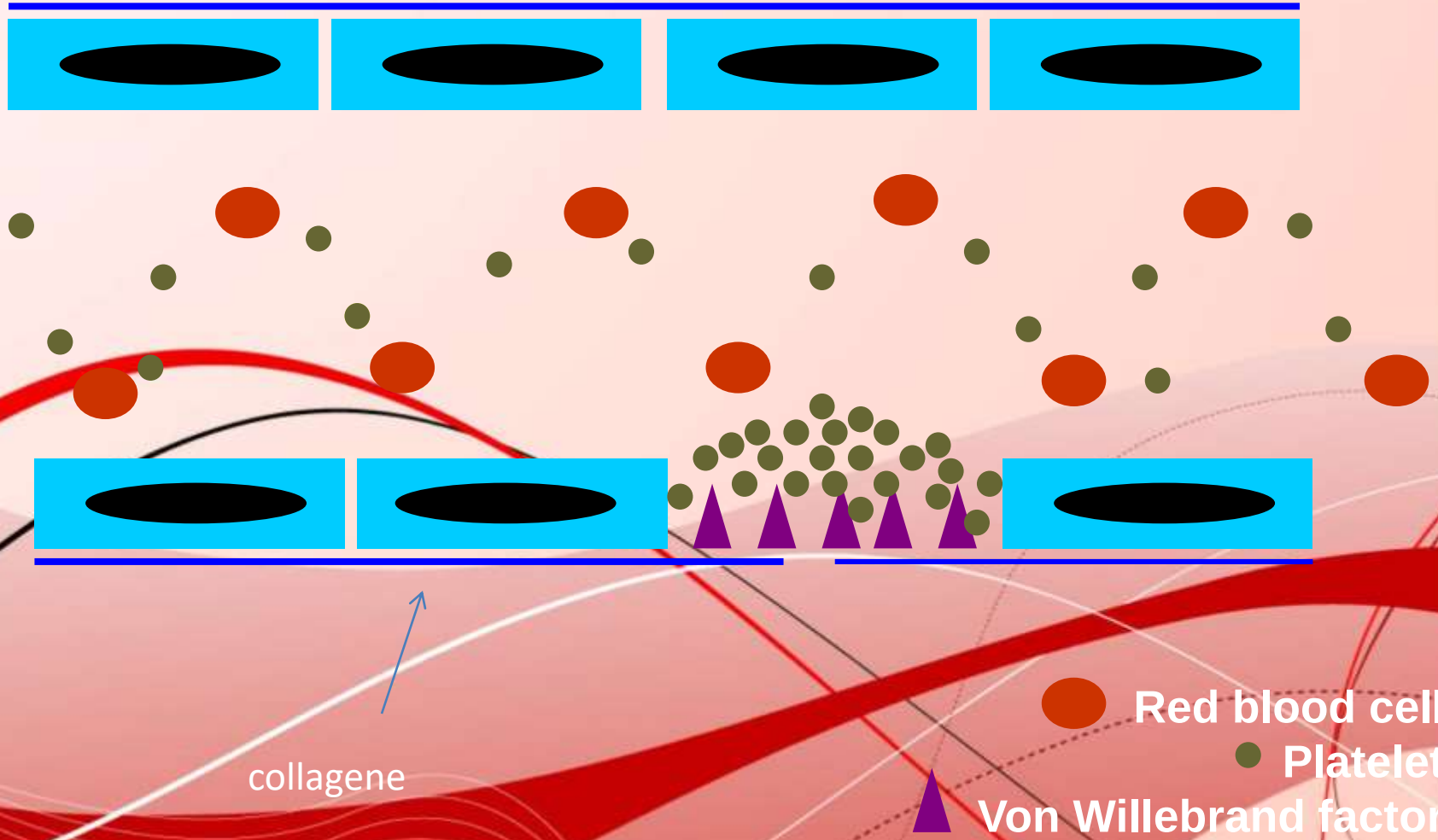
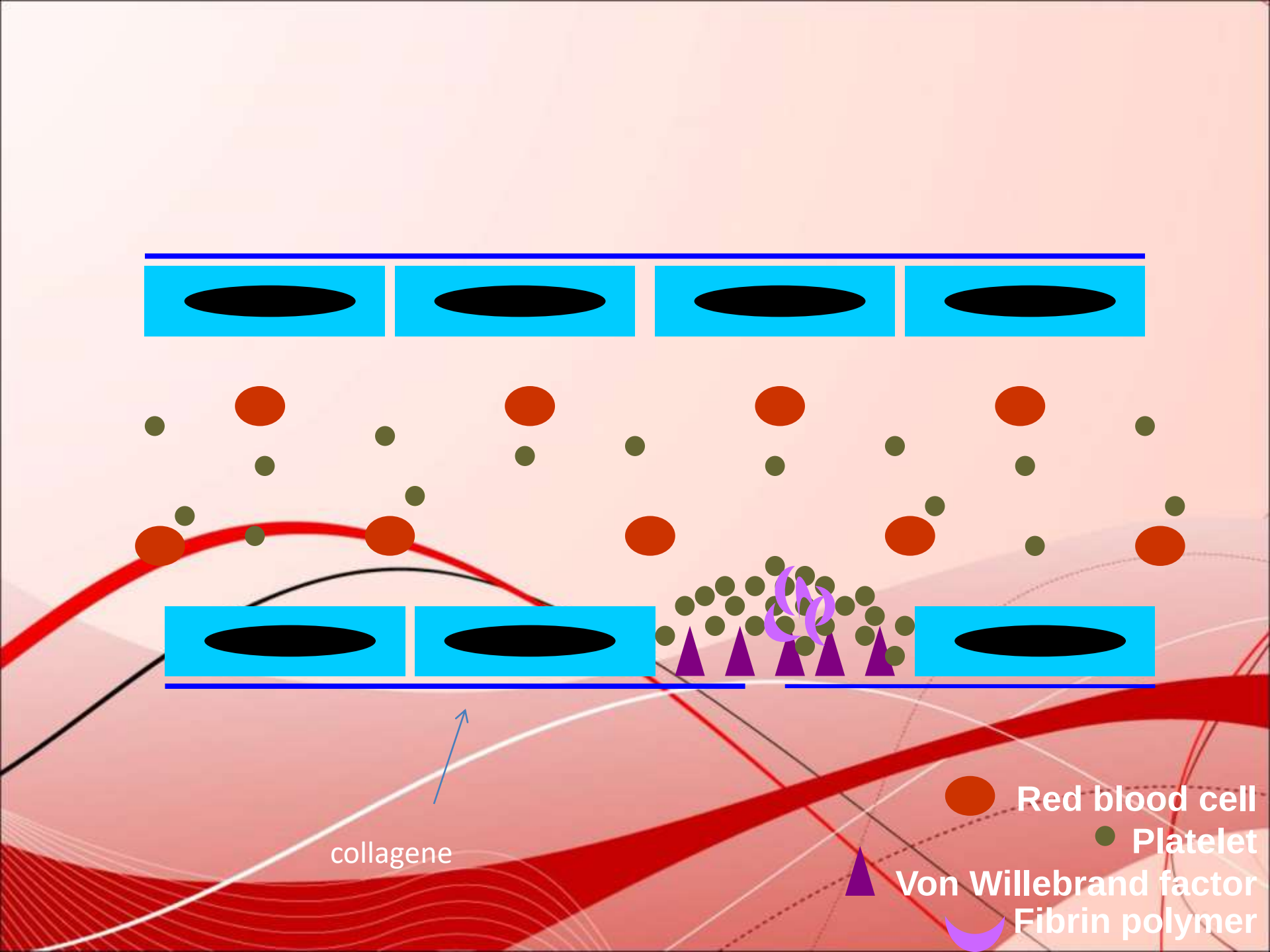
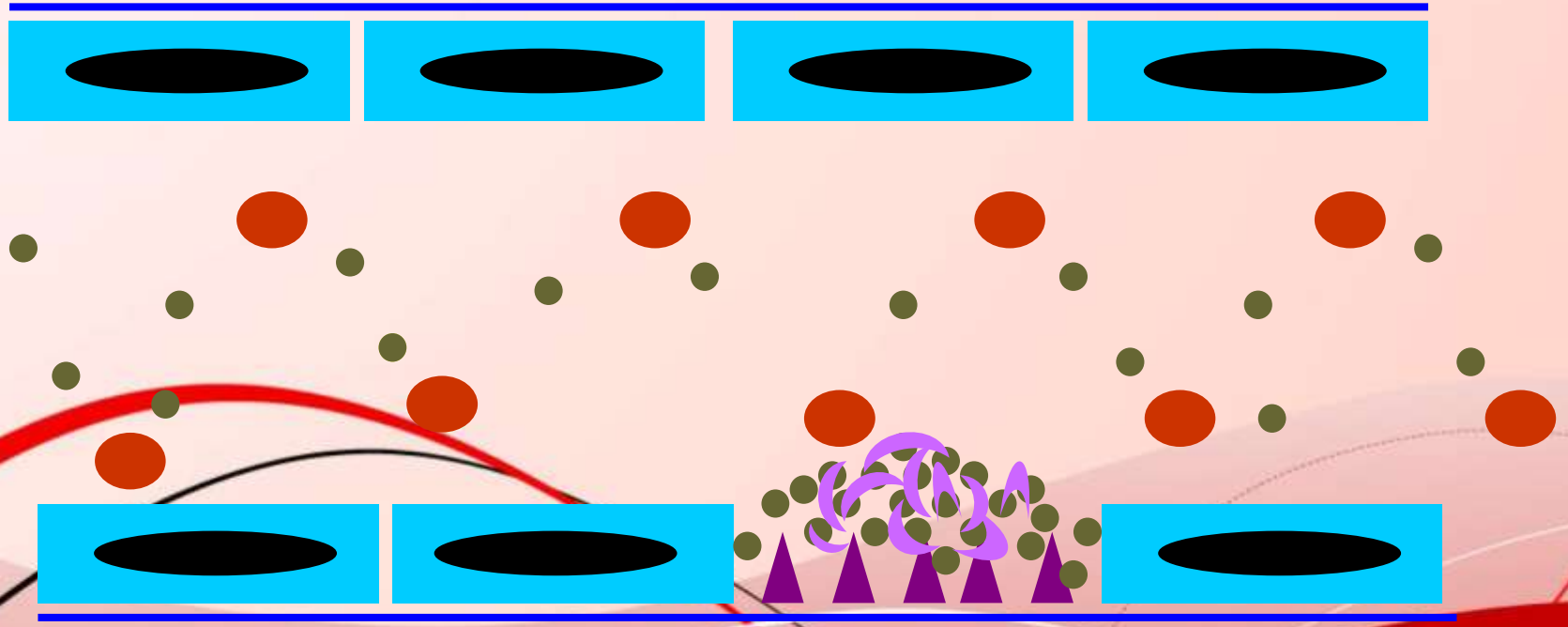
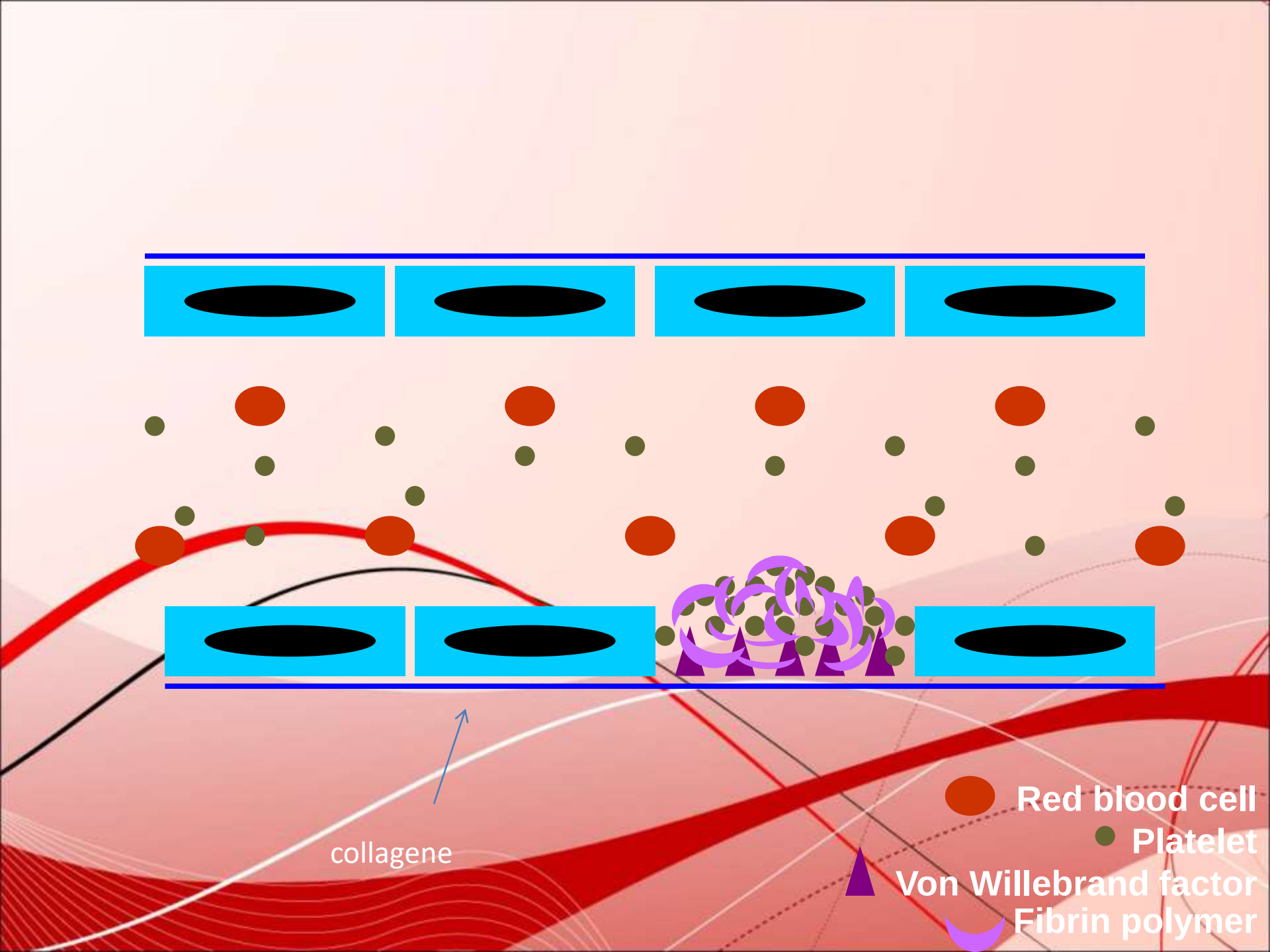
Red blood cell
Platelet
Von Willebrand factor

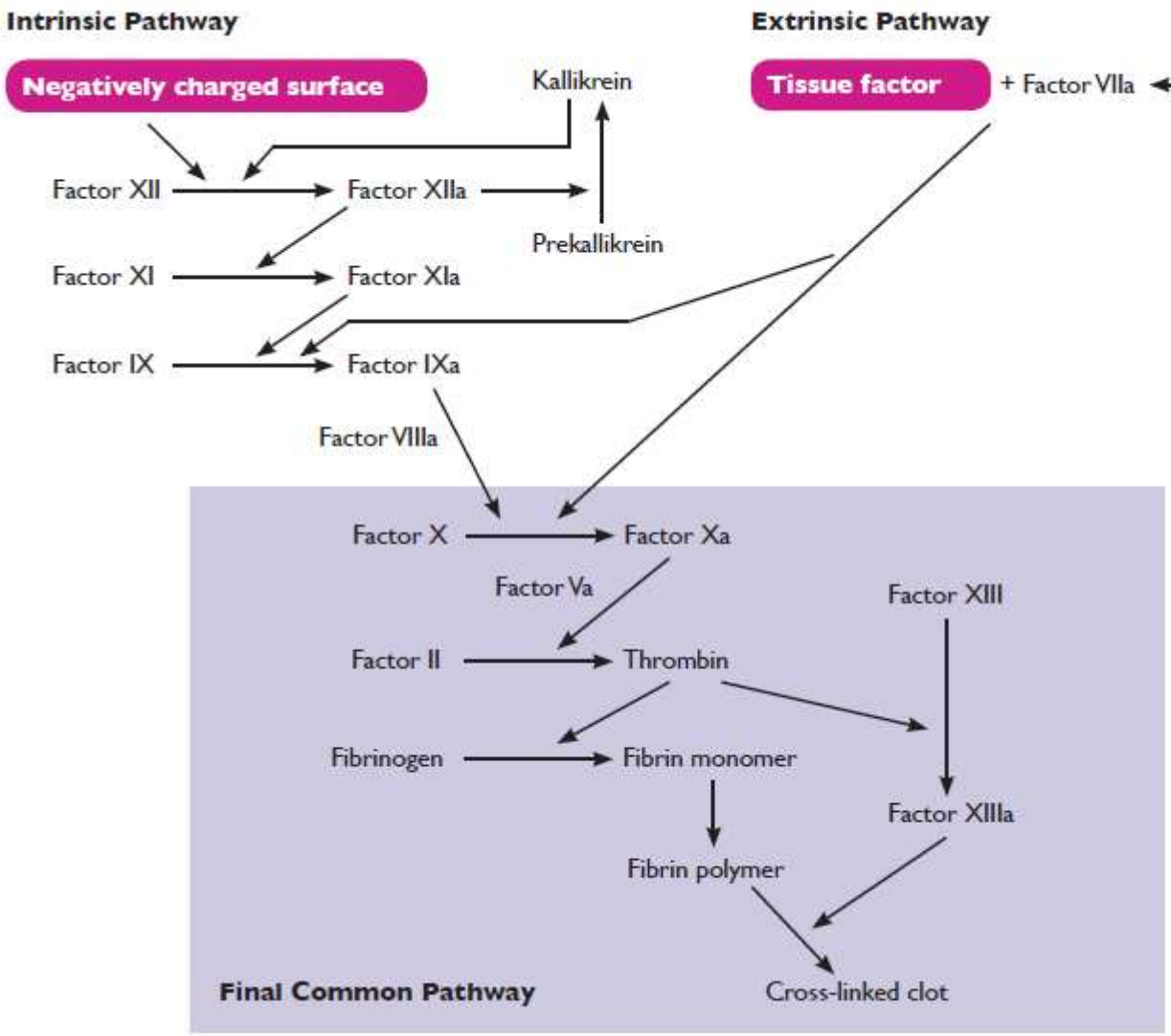

collagene
Red blood cell
Platelet
Von Willebrand factor


collagene
Red blood cell
Platelet
Von Willebrand factor
Fibrin polymer


collagene
Red blood cell
Platelet
Von Willebrand factor
Fibrin polymer

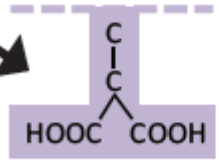

collagene
Red blood cell
Platelet
Von Willebrand factor
Fibrin polymer

Coagulation factors



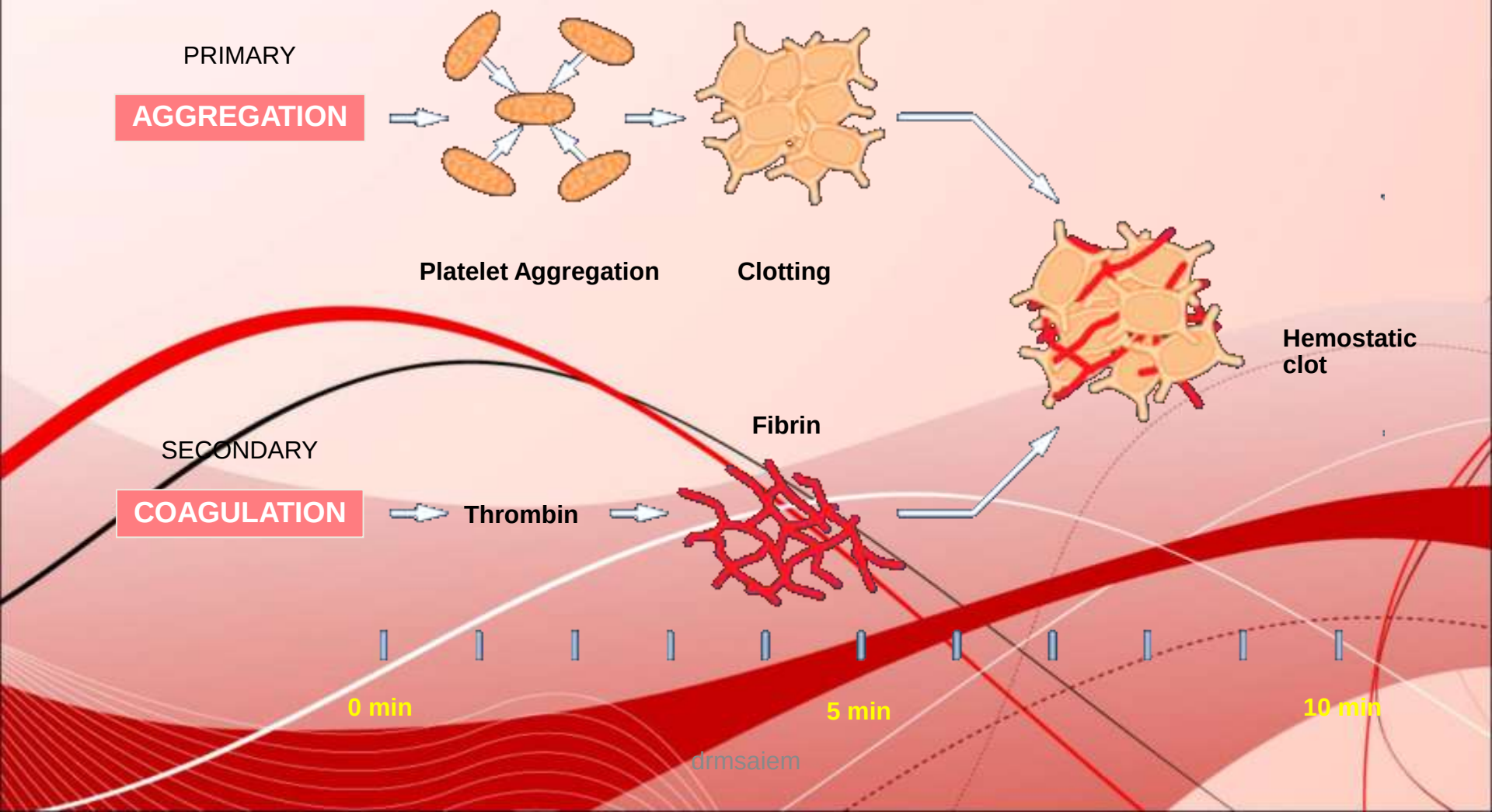
Vitamin K-dependent factor zymogen (before posttranslation modification)

Glu residues



Gla residues with phospholipid binding activity

Hemostatic Plug Formation



NATURAL INHIBITORS OF THE COAGULATION CASCADE

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

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)

Plasminogen/Plasmin

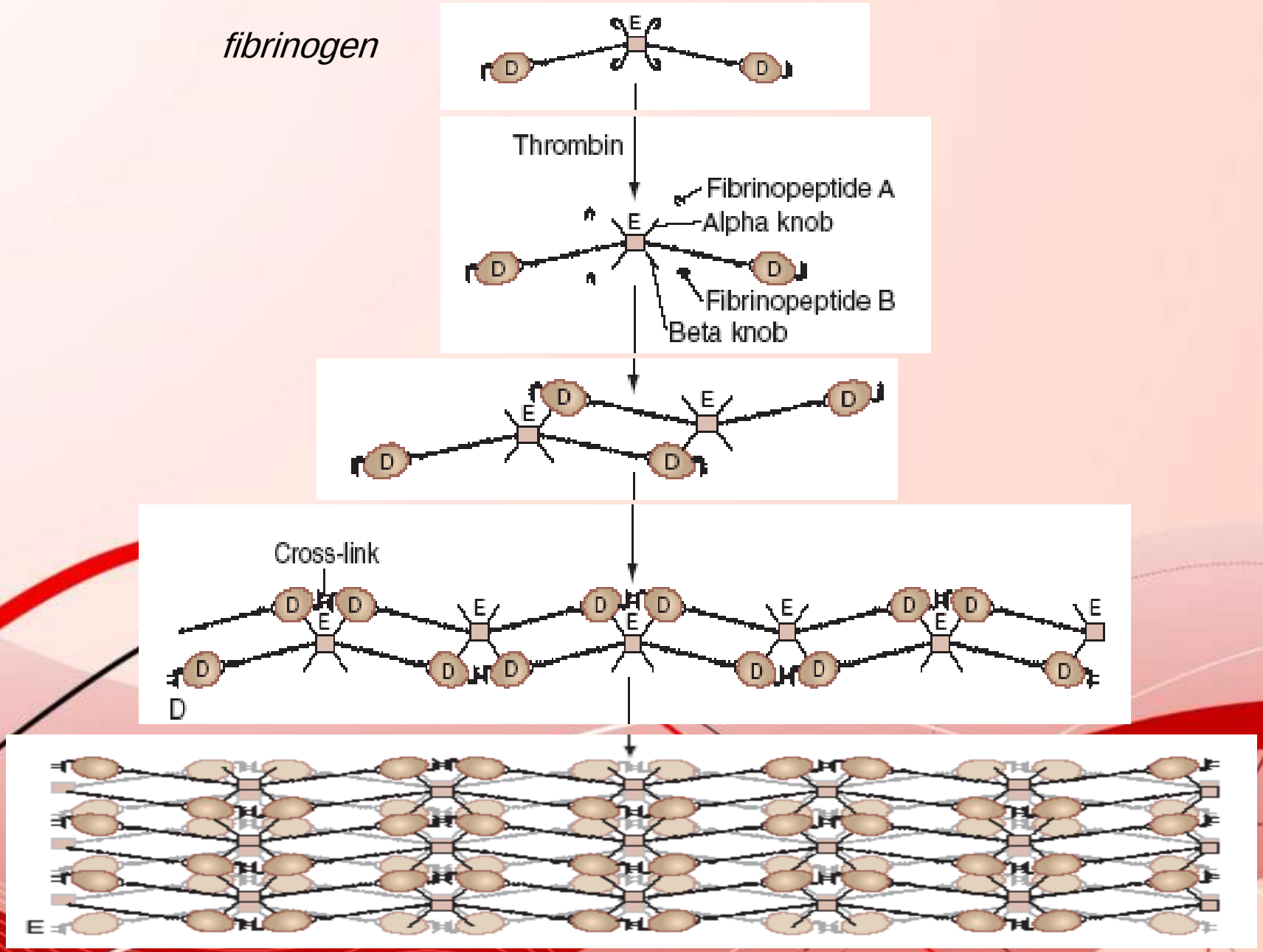
- *Plasmin is the enzyme that digests fibrin and thus dissolves clots.*

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
D
E
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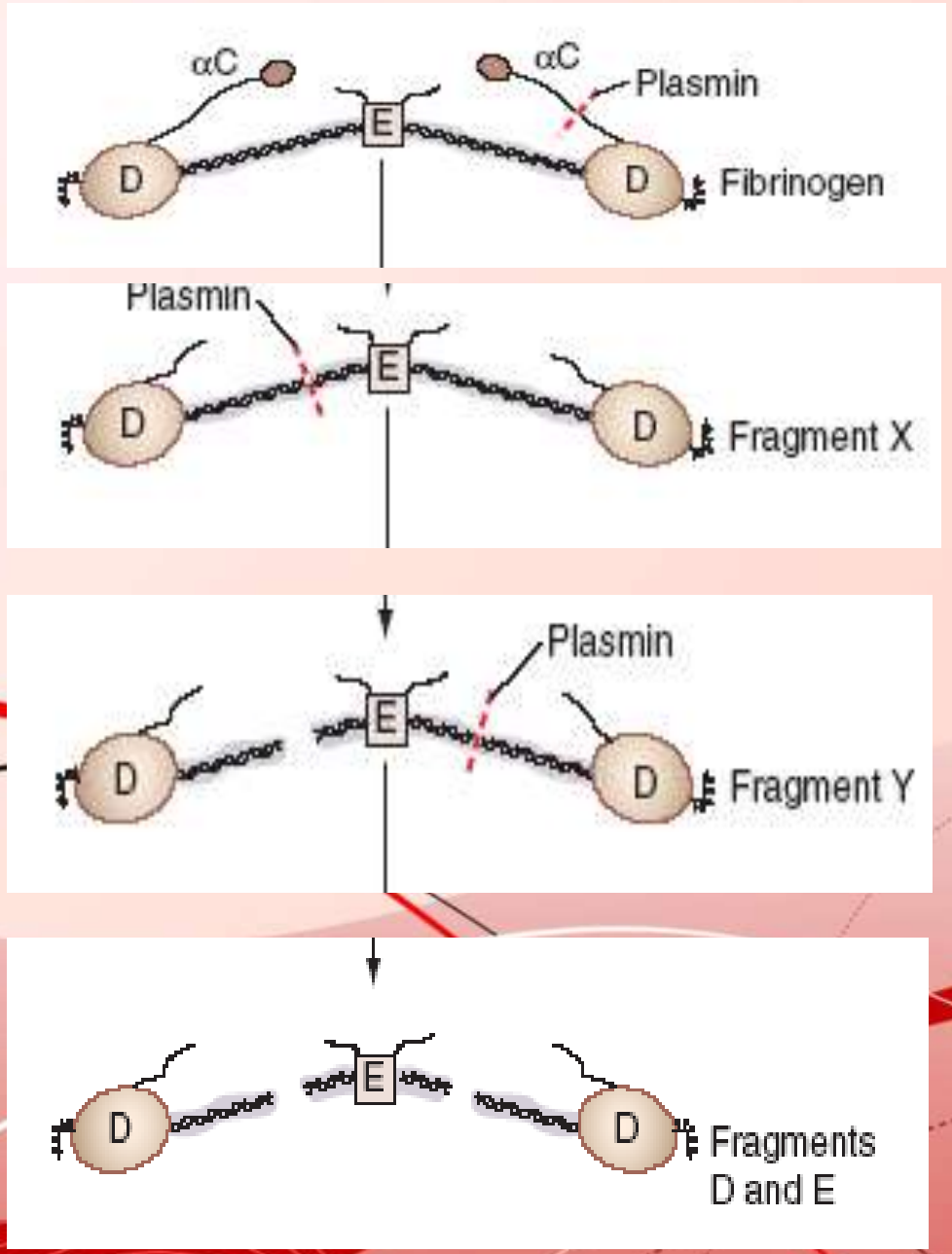
D
E

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

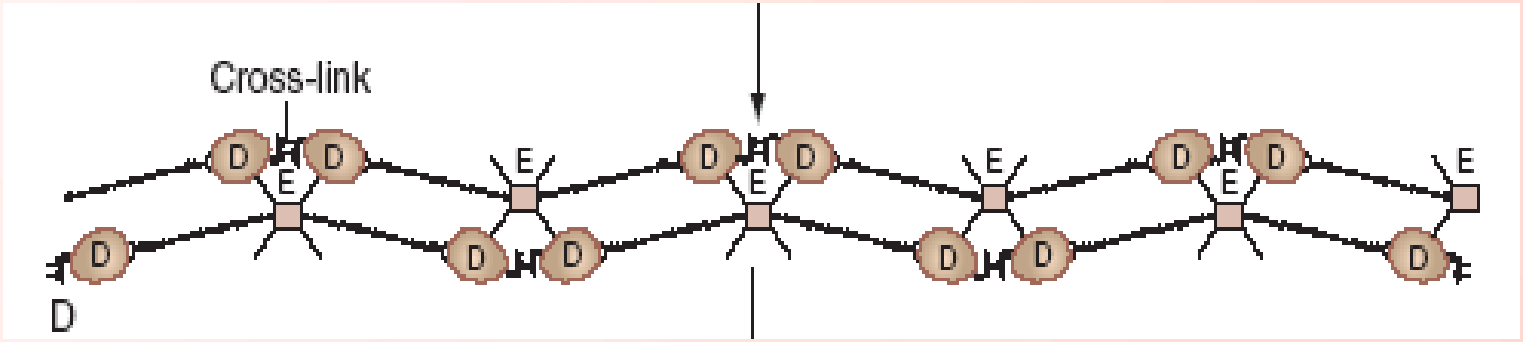
- A specific fibrin degradation product
- results from the digestion of fibrin that has been crosslinked
- by factor XIIIa.
- **Thus, the presence of D-dimer in circulation indicates that:**

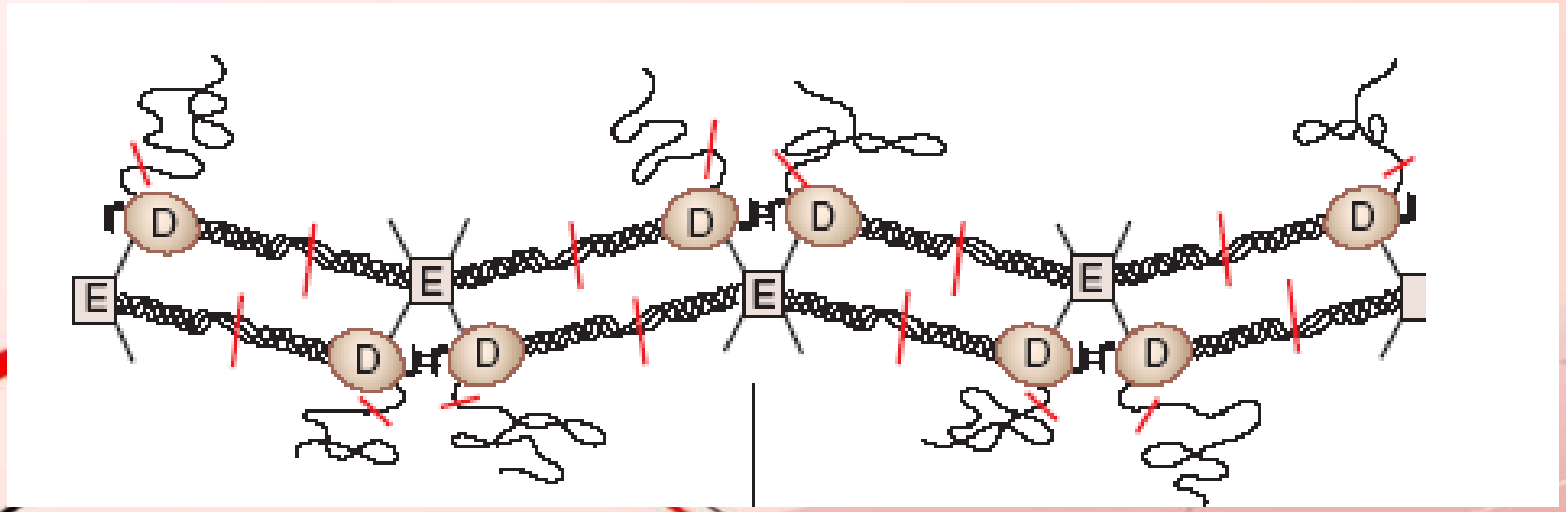
✓ thrombin has been activated and has resulted in both **fibrin clotting**

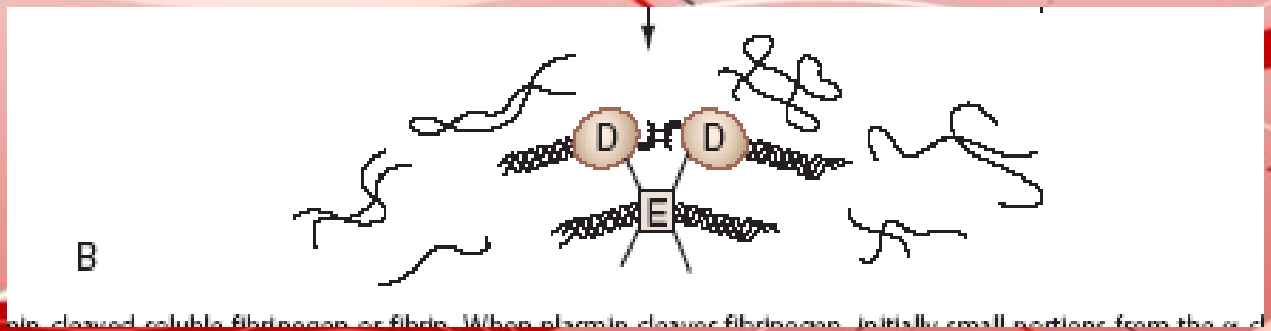
✓ activation of factor XIII to XIIIa,

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

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







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

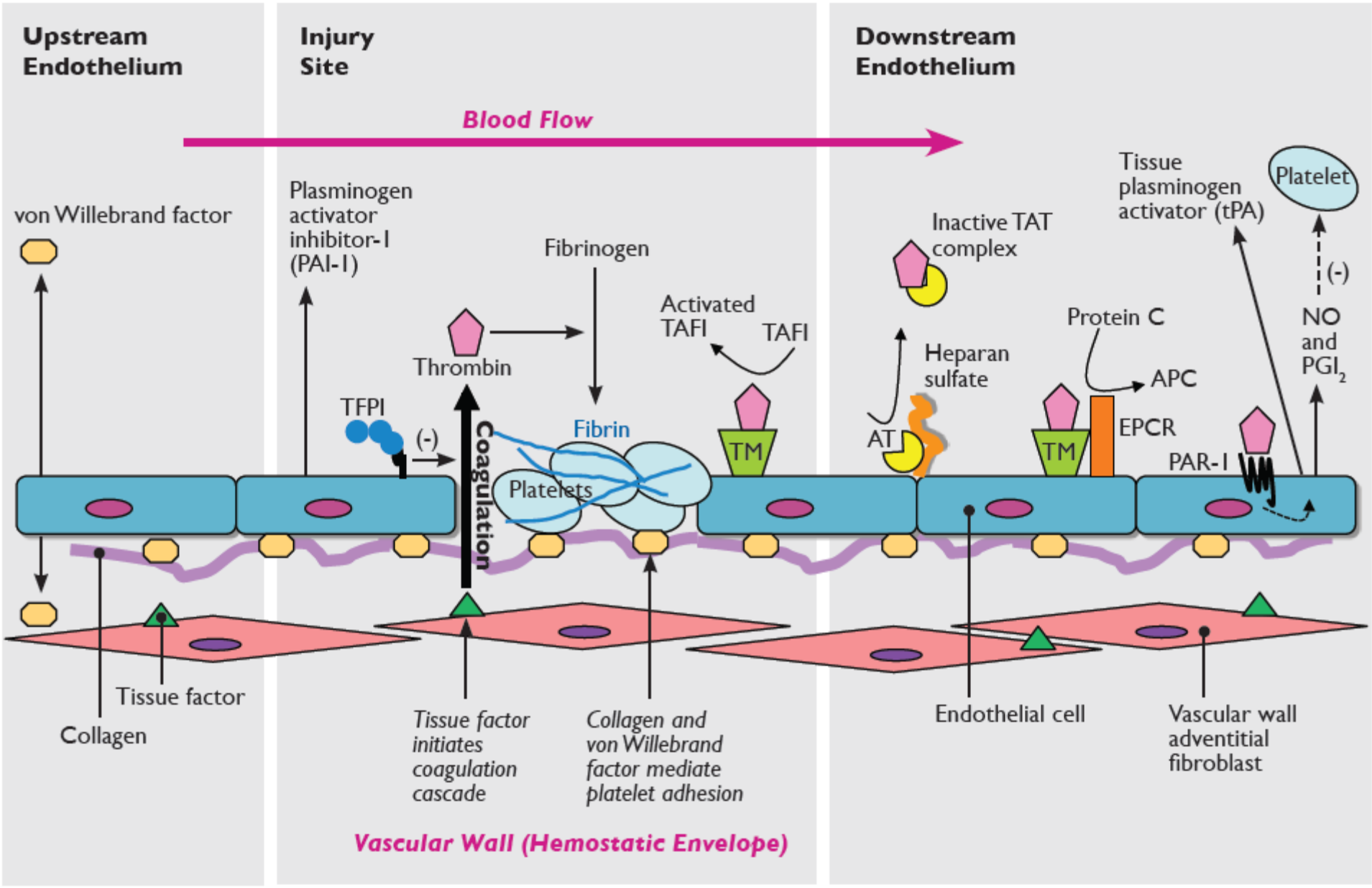

Upstream Endothelium
Injury Site
Downstream Endothelium
Blood Flow
von Willebrand factor
Plasminogen activator inhibitor-1 (PAI-1)
Fibrinogen
Thrombin
Activated TAFI
TAFI
TFPI
(-)
Coagulation
Fibrin
Platelets
TM
Inactive TAT complex
Heparan sulfate
AT
Protein C
APC
TM
EPCR
PAR-1
Tissue plasminogen activator (tPA)
Platelet
(-)
NO and PGI_2
Tissue factor
Collagen
Tissue factor initiates coagulation cascade
Collagen and von Willebrand factor mediate platelet adhesion
Endothelial cell
Vascular wall adventitial fibroblast
Vascular Wall (Hemostatic Envelope)

Table 1: Thrombogenic and antithrombogenic components in the body		
Site	**Thrombogenic**	**Antithrombogenic**
Vessel wall	Exposed endothelium	Heparin
	TF	Thrombomodulin
	Collagen	Tissue plasminogen activator
Circulating elements	Platelets	Antithrombin
	Platelet activating factor	Protein C and S
	Clotting factor	Plasminogen
	Prothrombin	
	Fibrinogen	
	vWF	

vWF – Von Willebrand factor; TF – Tissue factor

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

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

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

- HELLP syndrome
  - High blood pressure
  - Elevated liver enzymes
  - Low platlets
  - Occurs in pregnancy and is life-threatening
  - If the CBC of a woman in labor shows schistocytes and low platlets alert the physician immediately – the baby must be delivered immediately to save the mom!

Vitamin K Deficiency

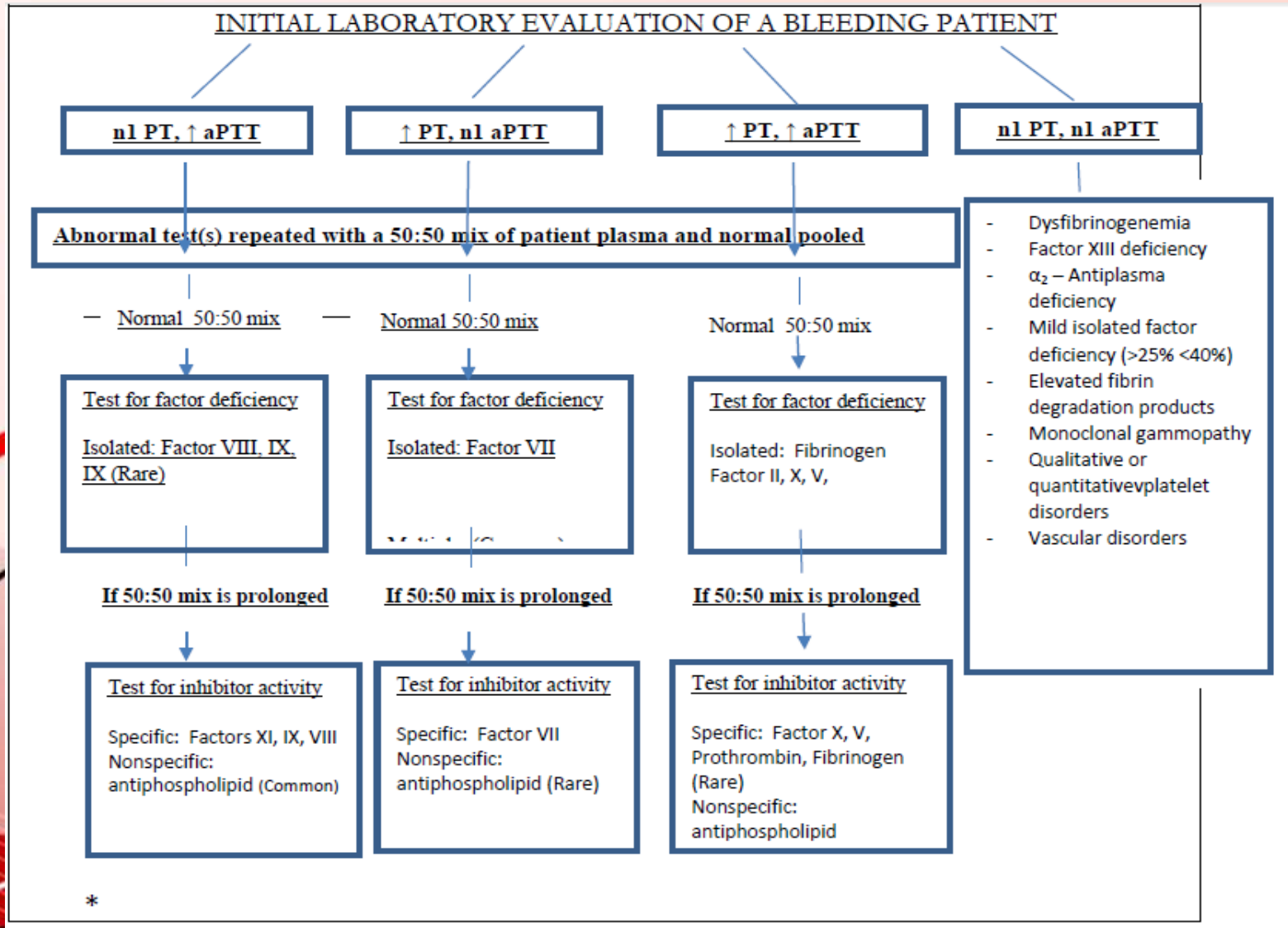
- Precursor proteins synthesized by hepatocytes
  - Not γ-carboxylated
  - Ca++-binding sites are nonfunctional
  - Induced functional deficiencies of all vitamin-K dependent proteins
  - Causes of vitamin K deficiency in adults
    - Malabsorptive syndromes
    - Biliary tract obstruction
    - Prolonged broad-spectrum antibiotics
- Most often seen in newborns
  - Hemorrhagic disease of the newborn
    - Due to newborn hepatic immaturity

Table 5: Classification of disorders of coagulation

Bleeding disorders	Thrombotic disorders (thrombophilia)
Hereditary	Hereditary
Von Willebrand disease	Hereditary thrombophillia
Haemophilia A	Antithrombin III deficiency
Haemophilia B	Protein C deficiency
Haemophilia C	Protein S deficiency
Factor V deficiency	Factor V Leiden (factor V mutation)
Factor X deficiency	Prothrombin mutation (Gene 20210A mutation)
Factor VII deficiency	
Factor XIII deficiency	
Prothrombin deficiency	
Afibrinogenemia	
Acquired	Acquired
Consumptive coagulopathies	Antiphospholipid antibody syndrome
DIC	Increased levels of factors VIII, IX, XI, or fibrinogen
Microangiopathic haemolytic anemias	Fibrinolysis defects
Vitamin K deficiency	Homozygous homocystinuria
Liver disease	

DIC – Disseminated intravascular coagulation

How to Evaluate the Screening Coagulation Tests in a Bleeding Patient



Trigger to order Mixing Study

- Prolonged PT or PTT
- When Mix ordered on PTT lab first does Hepzyme:
- If PTT normalizes to PTT normal range, no further testing
- **Important Premises for Mixing Study:**
- The results of the mix study will allow one to distinguish
- Between Factor deficiency or an inhibitor
- Theoretically the normal level of coagulation factor
- Present in Normal Plasma will be enough to correct the deficit of factor (S)
- In patient plasma
- PTT or PT reagents should be sensitive to factor levels approximating 30-40% activity
- An inhibitor present in patient plasma will act against not only its own coagulation factors but
- Also against those in normal plasma.

- Detecting inhibitors having various avidities and concentration is dependent upon use of
- different mix types 1:1 or 4:1
- 4:1 may be more useful when initial PTT is only mildly elevated
- effective in picking up weaker inhibitors
- may be better to detect the lupus anticoagulant in the presence of warfarin
- caveat is that the 4:1 mix may not correct for a mild factor and thereby not differentiate between this and a mild inhibitor
- heparin in the sample produces non predictable mix results in terms of correction
- Extending incubation times immediate versus 1-2 hours
- Inhibitors such as Factor V III inhibitors and 15% of lupus anticoagulantas require time to exert their effect

Some General Recommendations for Hemostais Testing
Note IV

Recommended Diagnostic Testing for Thrombotic and Bleeding Disorders	
Clinical Condition	Recommended Initial Tests
Venous Thromboembolism*	APC Resistance Assay (if Positive Factor V Leiden DNA testing). Factor II gene mutation, PTT, dRVVT with Confirm, Anticardiolipin and beta-2 Glycoprotein I antibody testing, Protein C, Protein S, Antithrombin Activities (II levels, homoocysteine)
Arterial Thromboembolism	PTT, dRVVT with Confirm, Anticardiolipin and beta-2 Glycoprotein I antibody testing, Homocysteine, Thrombin time, Fibrinogen, Reptilase assay
Platelet-type (mucocultaneous) bleeding-defect in primary hemostasis	Platelet count, Peripheral blood smear, PFA-100 assay Bleeding time (?), vWF Antigen, Ristocetin Cofactor assay, Factor VIII activity (Platelet aggregation,)
Coagulation factor-type bleeding (e.g., hemarthroses, deep tissue hematoma)-defect in secondary hemostasis	PT, PTT, Thrombin time, Fibrinogen, Factor assays (depending upon screening test results)
Delayed bleeding	Factor XIII screen (and factor XIII activity depending upon level of suspicion), α2-antiplasmin, Plasminogen activator inhibitor 1 (PAI-l)