Sudan Symex Days II

Platelets, blood coagulation and haemostasis

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- Normal haemostatic response to vascular damage depends on the interaction between: the blood vessel wall, circulating platelets and blood coagulation factors.
- Haemostatic system represents the balance between procoagulant and anticoagulant mechanisms.
- The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels.

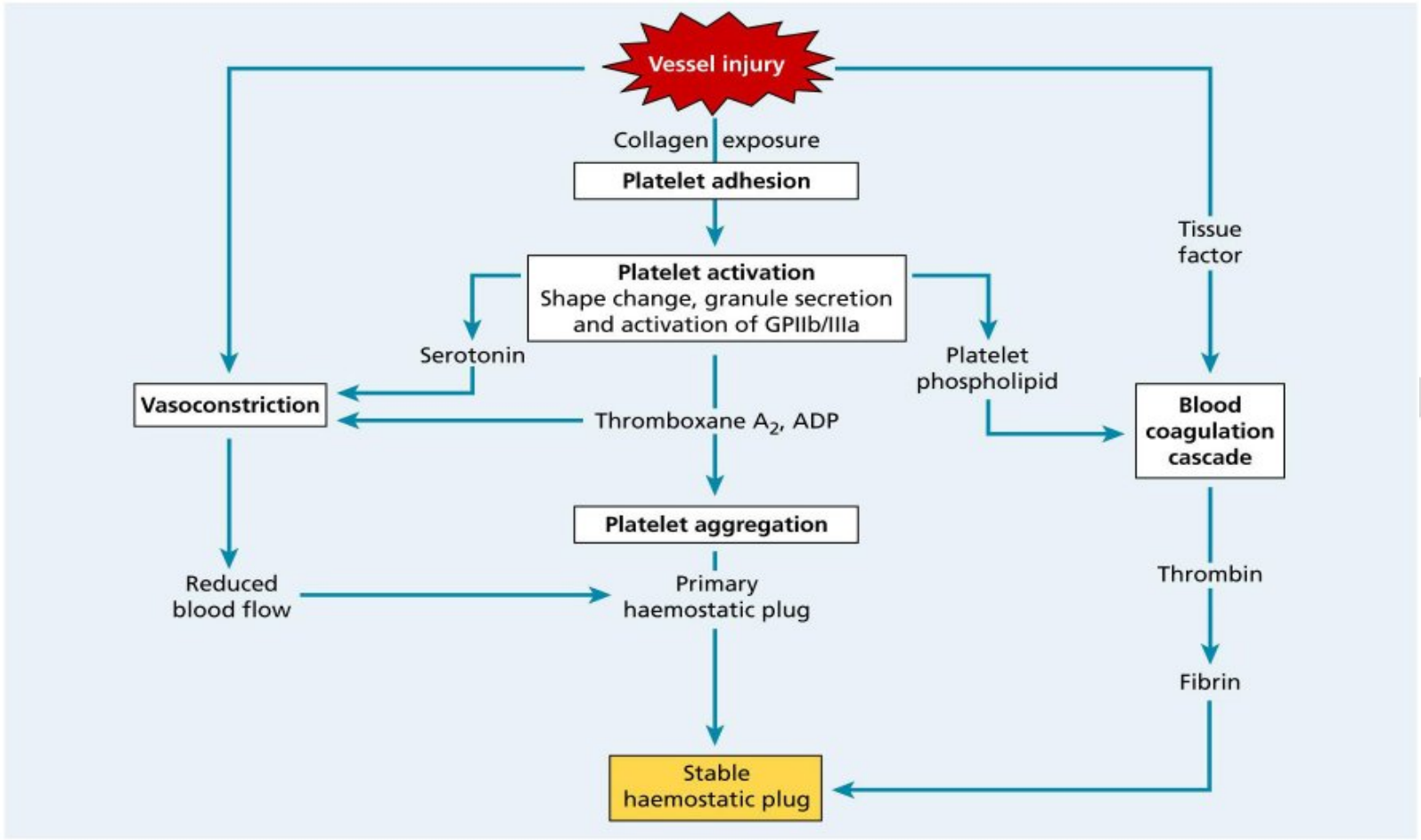

Figure 24.1 The involvement of blood vessels, platelets and blood coagulation in haemostasis. ADP, adenosine diphosphate.

Components of haemostatic response

- **Platelets:**
- Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes.
- Megakaryoblast arises by a process of differentiation from the mixed myeloid progenitores (CFU-GEMM).

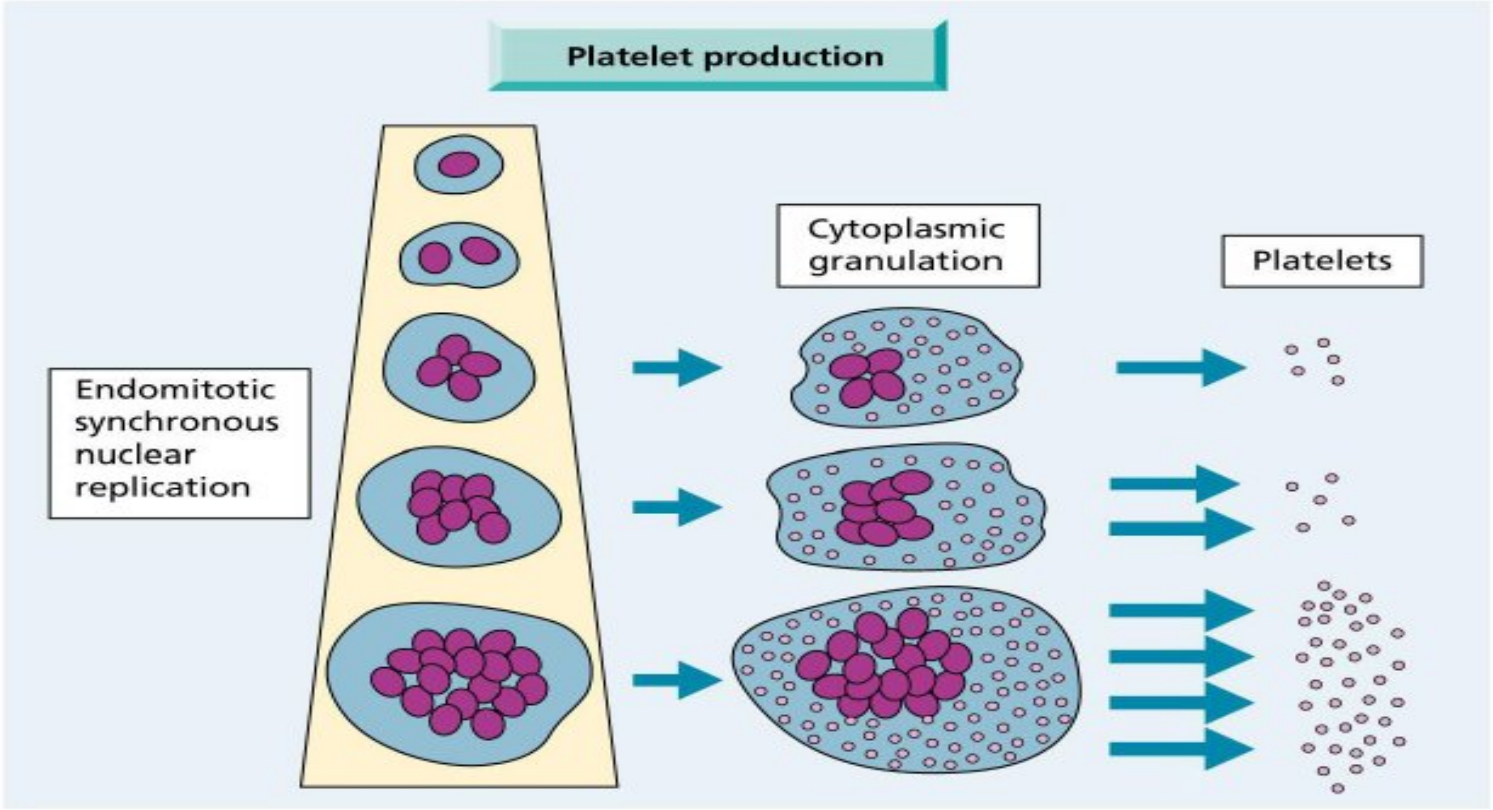

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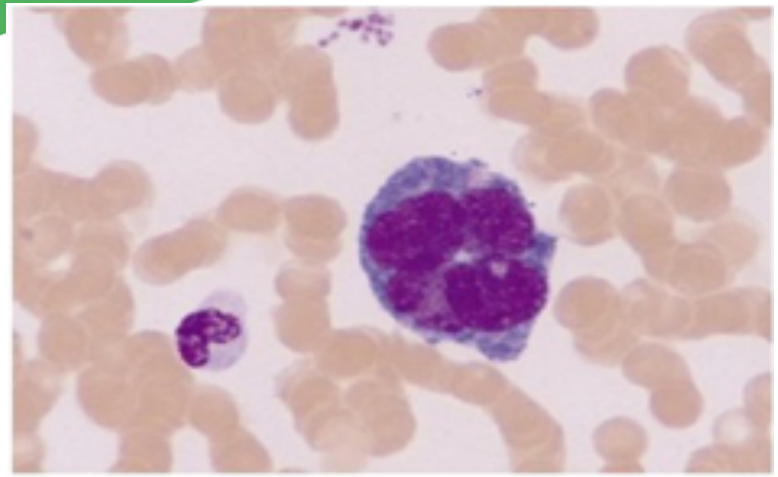
Figure 24.2 Simplified diagram to illustrate platelet production from megakaryocytes.

- The time interval from differentiation of the human stem cell to the production of platelets averages approximately 10 days.
- Thrombopoietin is the major regulator of platelet production.

- The normal platelet count is (150-400 x 10^9/L).
- The normal platelet lifespan is 7-10 days.
- Up to one-third of the marrow output of platelets is trapped in the normal spleen.
- This rises to 90% in cases of massive splenomegaly.



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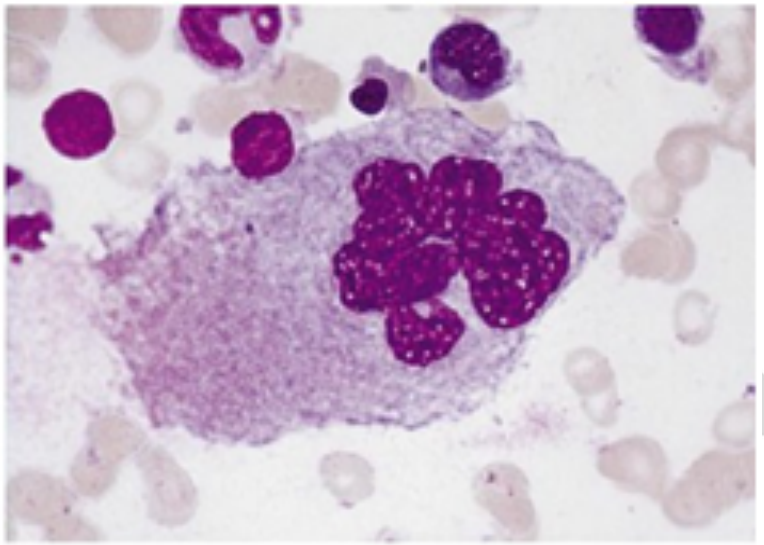

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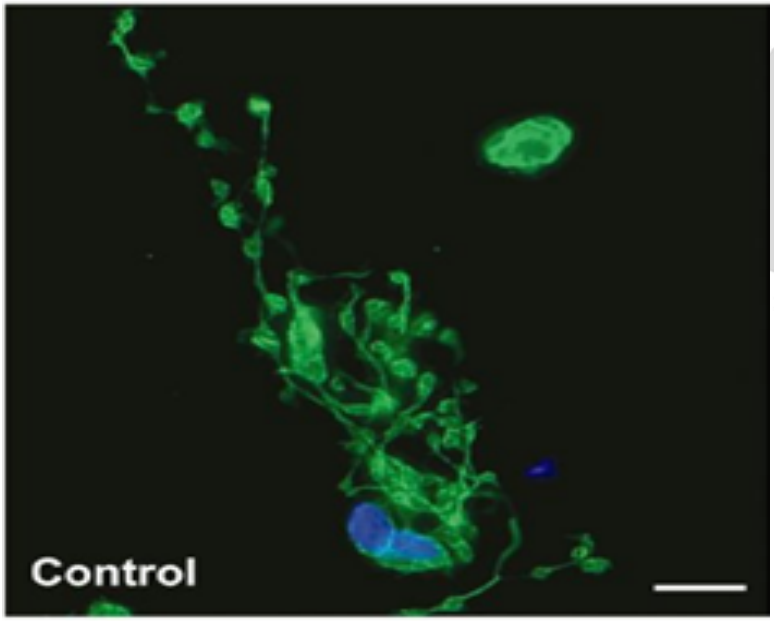


(c)

Figure 24.3 Megakaryocytes: **(a)** immature form with basophilic cytoplasm; **(b)** mature form with many nuclear lobes and pronounced granulation of the cytoplasm. **(c)** Megakaryocyte in culture, stained for α-tubulin (green). Proplatelets can be seen budding from the tips of megakaryocyte cytoplasm. (From Pecci A. et al. (2009) *Thrombosis and Haemostasis* **109**, 90–96, with permission; with thanks to Professor Pecci for Fig. 24.3c.)

Platelet structure

- Platelets are extremely small and discoid, 3.0 x 0.5 nm in diameter, with a mean volume of 7-11 fL.
- Glycoproteins of the surface coat are important in the platelet adhesion and aggregation.

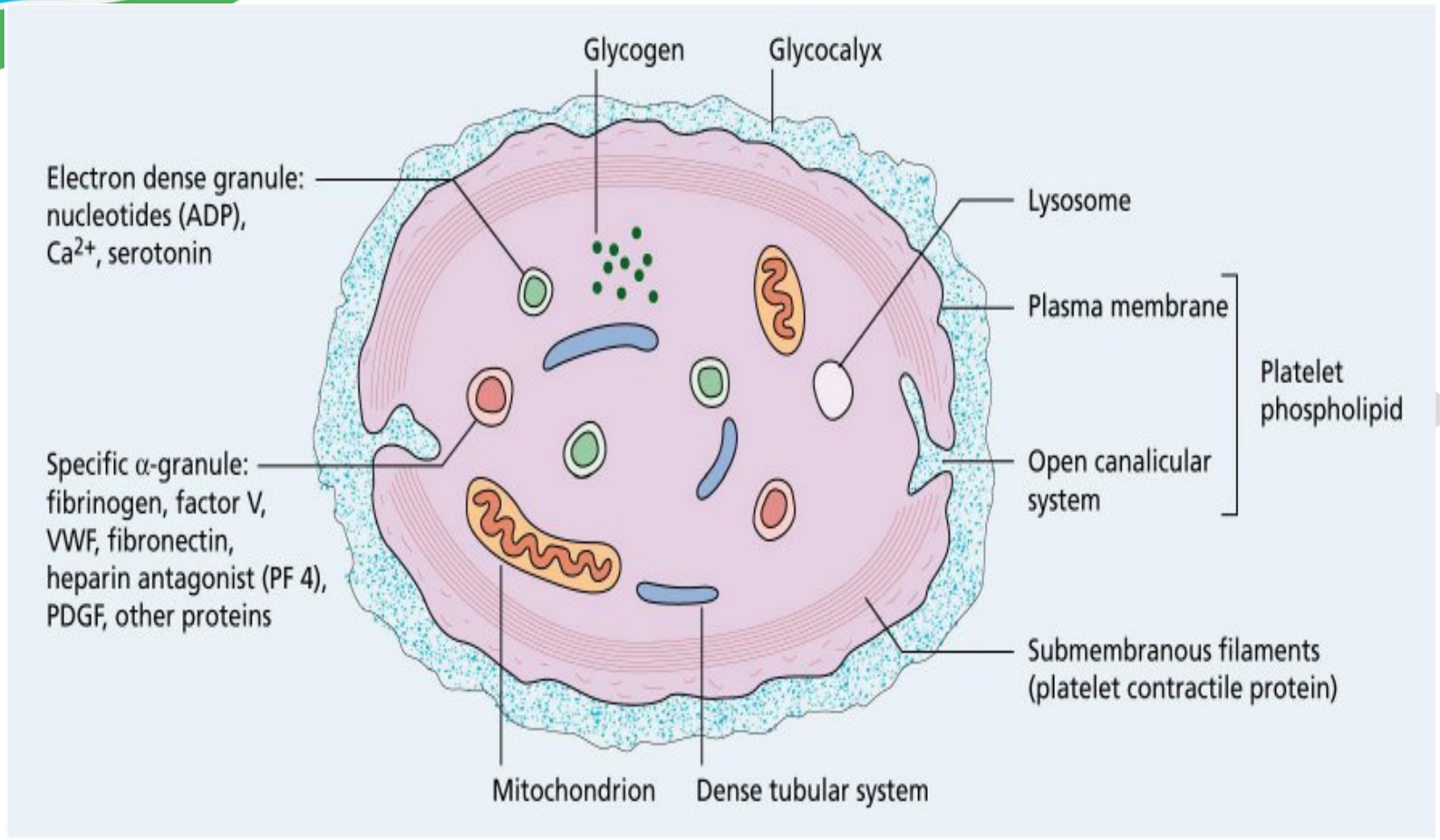


Figure 24.4 The ultrastructure of platelets. ADP, adenosine diphosphate; PDGF, platelet-derived growth factor; PF, platelet factor; VWF, von Willebrand factor.

Platelet function

- The main function of platelets is the formation of mechanical plugs during the normal haemostatic response to vascular injury.
- The immobilization of platelets at the sites of vascular injury requires specific platelet-vessel wall (adhesion) and platelet-platelet (aggregation) interactions.

- The blood flow conditions determine the specific receptor ligand interactions.

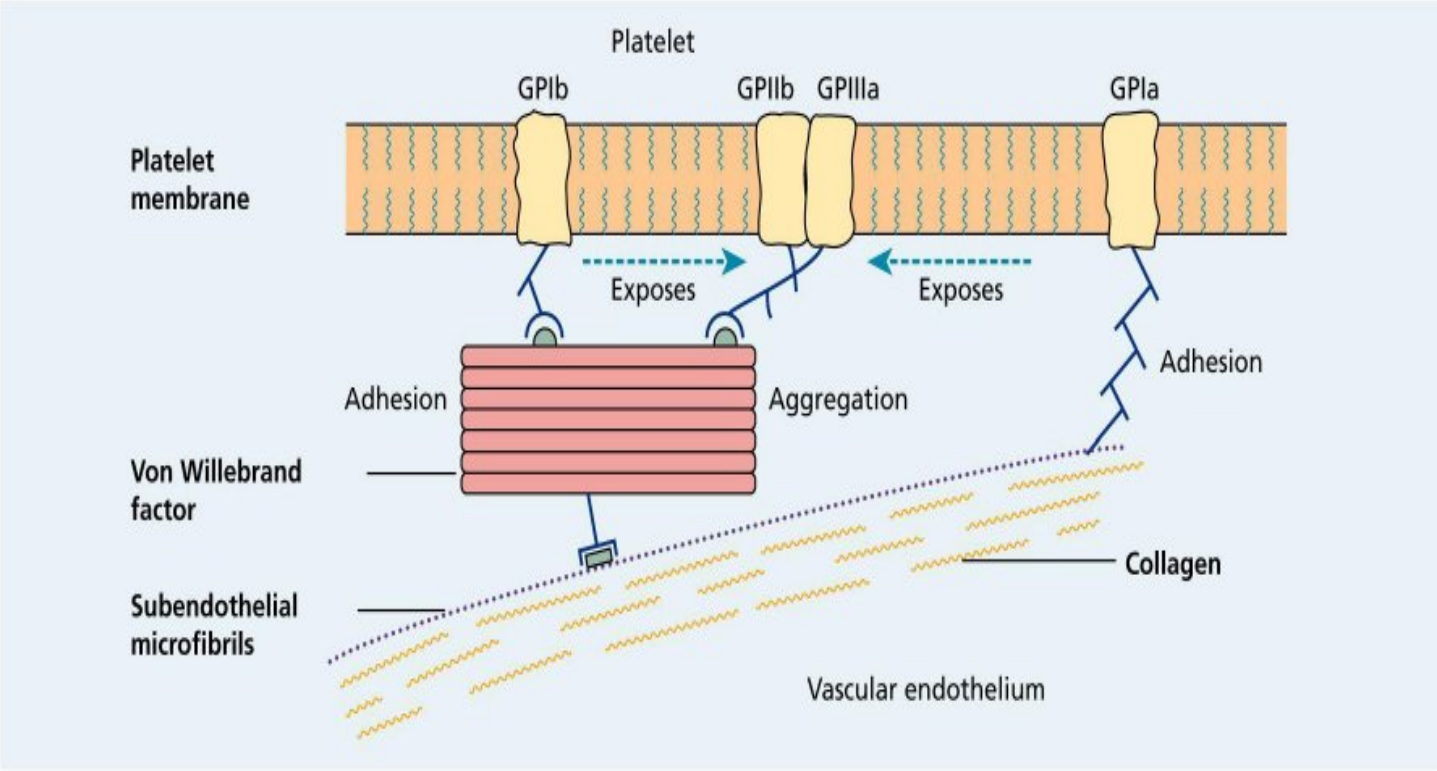


Figure 24.5 Platelet adhesion. The binding of glycoprotein (GP) Ib (which consists of four proteins: GPIbα, GPIbβ, GPIX, GPV) to von Willebrand factor leads to adhesion to the subendothelium and also exposes the GPIIb/IIIa ($\alpha_{IIb}\beta_3$ integrin) binding sites to fibrinogen and von Willebrand factor leading to platelet aggregation. The GPIa site permits direct adhesion to collagen and also explores the GPIIb/IIIa binding site.

- ά-Granule contents play an important role in platelet aggregate formation and stabilization.
- ADP and TXA_2 , released from dense granules, play a major positive feedback role in promoting platelet activation.

Platelet procoagulant activity

- Membrane phospholipid (platelet factor 3) is important for two reactions in the coagulation cascade.
- Both phospholipid-mediated reactions are calcium ion dependent.

- (Tenase) involves factors IXa, VIIIa and X and results in the formation of factor Xa.
- (Prothrombinase) involves factors Xa, Va and prothrombin (II) and results in the formation of thrombin.
- The phospholipid surface forms an ideal template for the crucial concentration and orientation of these proteins.

Natural inhibitors of platelet function

- Nitric oxide (NO) is released from endothelial cells, macrophages and platelets.
- It inhibits platelet activation and promotes vasodilatation.
- Prostacyclin is synthesized by endothelial cells.
- It inhibits platelet function and causes vasodilatation by raising (c-GMP) levels.

Endothelial cells

- They provides the basement membrane that normally separates collagen, elastin and fibronectin of the subendothelial connective tissue from the circulating blood.
- Loss or damage to the endothelium results in haemorrhage and activation of the haemostatic mechanism.

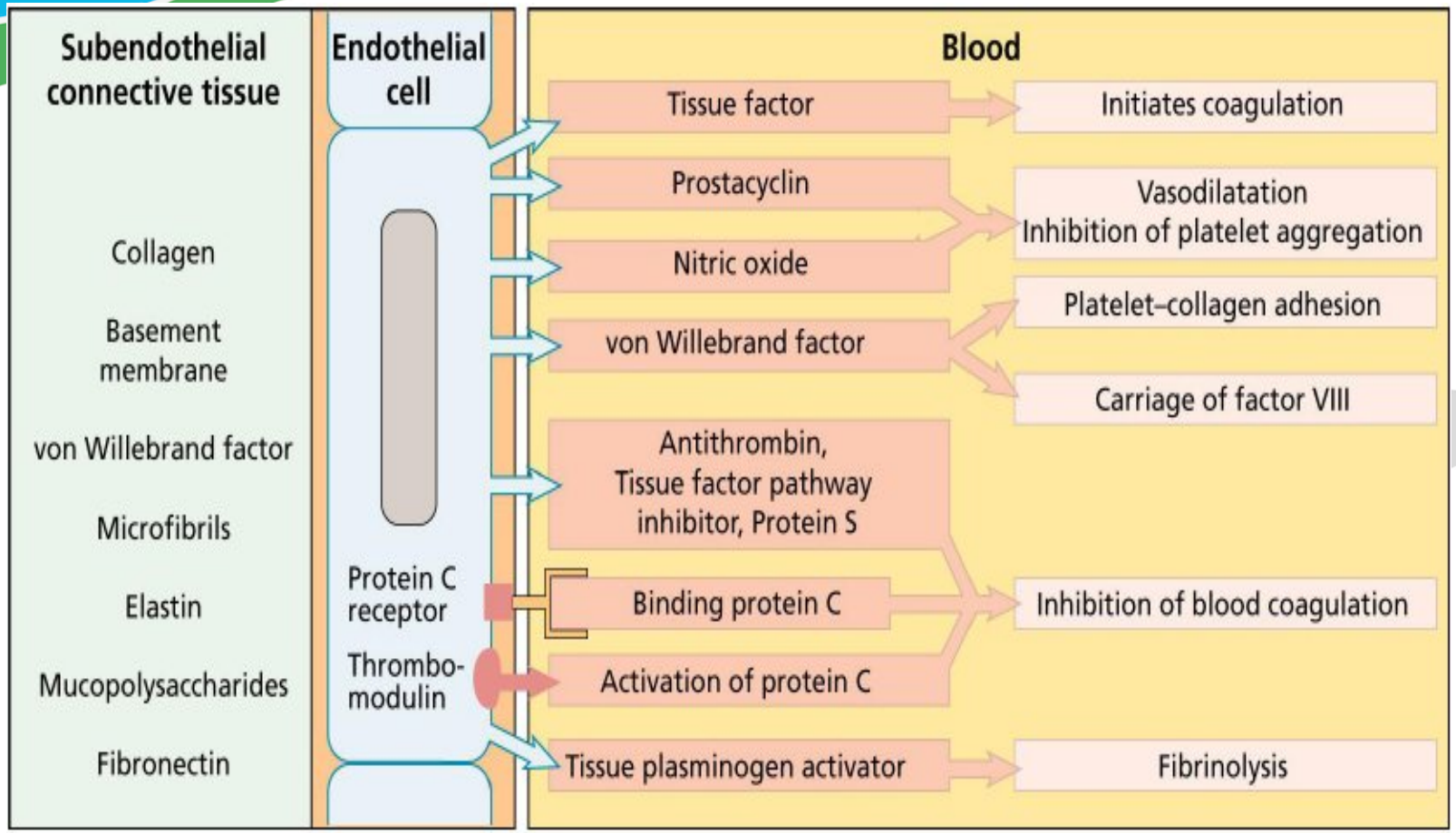


Figure 24.8 The endothelial cell forms a barrier between platelets and plasma clotting factors and the subendothelial connective tissues. Endothelial cells produce substances that can initiate coagulation, cause vasodilatation, inhibit platelet aggregation or haemostasis, or activate fibrinolysis.

Blood coagulation

- **The coagulation cascade:**
- Blood coagulation involves a biological amplification system in which relatively few initiation substances activate a cascade of coagulation factors enzymes → generation of thrombin → conversion of soluble plasma fibrinogen into fibrin.

- Fibrin enmeshes the platelet aggregates at the sites of vascular injury .
- It converts the unstable primary platelet plugs to firm, definitive and stable haemostatic plugs.
- With the exception of fibrinogen (fibrin clot subunit) the coagulation factors are either enzyme precursors or cofactors.

- Surface-mediated reactions occur on exposed collagen, platelet phospholipid and tissue factor.
- All the enzymes, except factor XIII (transglutaminase), are serine proteases.

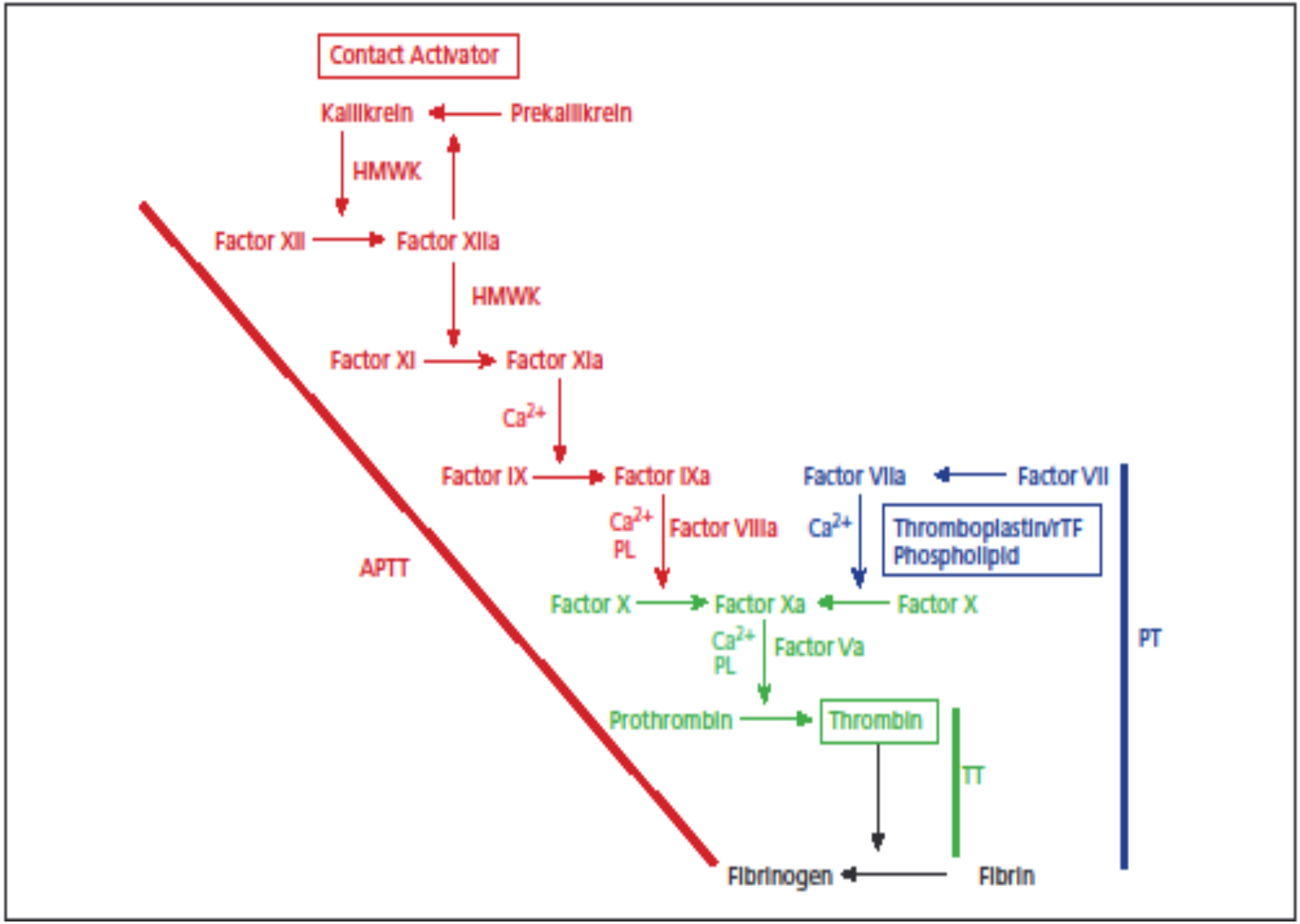
Table 24.1 The coagulation factors.

Factor number	Descriptive name	Active form
I	Fibrinogen	Fibrin subunit
II	Prothrombin	Serine protease
III	Tissue factor	Receptor/cofactor*
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart–Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman (contact) factor	Serine protease
XIII	Fibrin-stabilizing factor Prekallikrein (Fletcher factor) HMWK (Fitzgerald factor)	Transglutaminase Serine protease Cofactor*

HMWK, high molecular weight kininogen.
* Active without proteolytic modification.

Coagulation *in vivo*

- The traditional concept of blood coagulation was with separate intrinsic and extrinsic pathways → the common pathway with the generation of FXa.
- Each of them works independently.


Contact Activator
Kallikrein
Prekallikrein
HMWK
Factor XII
Factor XIIa
HMWK
Factor XI
Factor XIa
Ca2+
Factor IX
Factor IXa
Factor VIIa
Factor VII
Ca2+
PL
Factor VIIIa
Ca2+
Thromboplastin/rTF
Phospholipid
APTT
Factor X
Factor Xa
Factor X
Ca2+
PL
Factor Va
PT
Prothrombin
Thrombin
TT
Fibrinogen
Fibrin

- The traditional concept had been changed in that both pathways work in combination.
- The generation of thrombin following vascular injury occurs in two waves of different magnitude and functions (initiation and amplification).

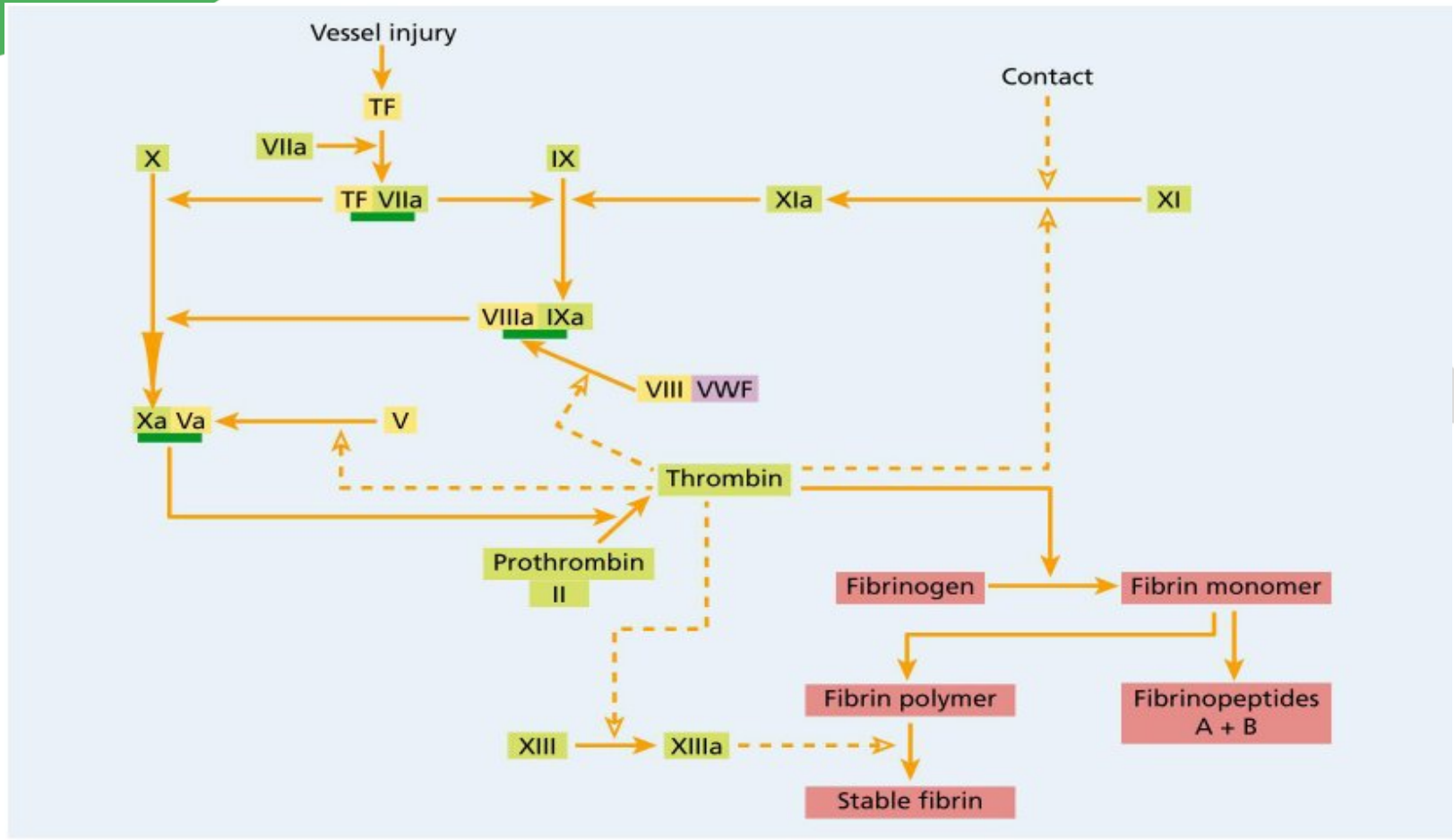


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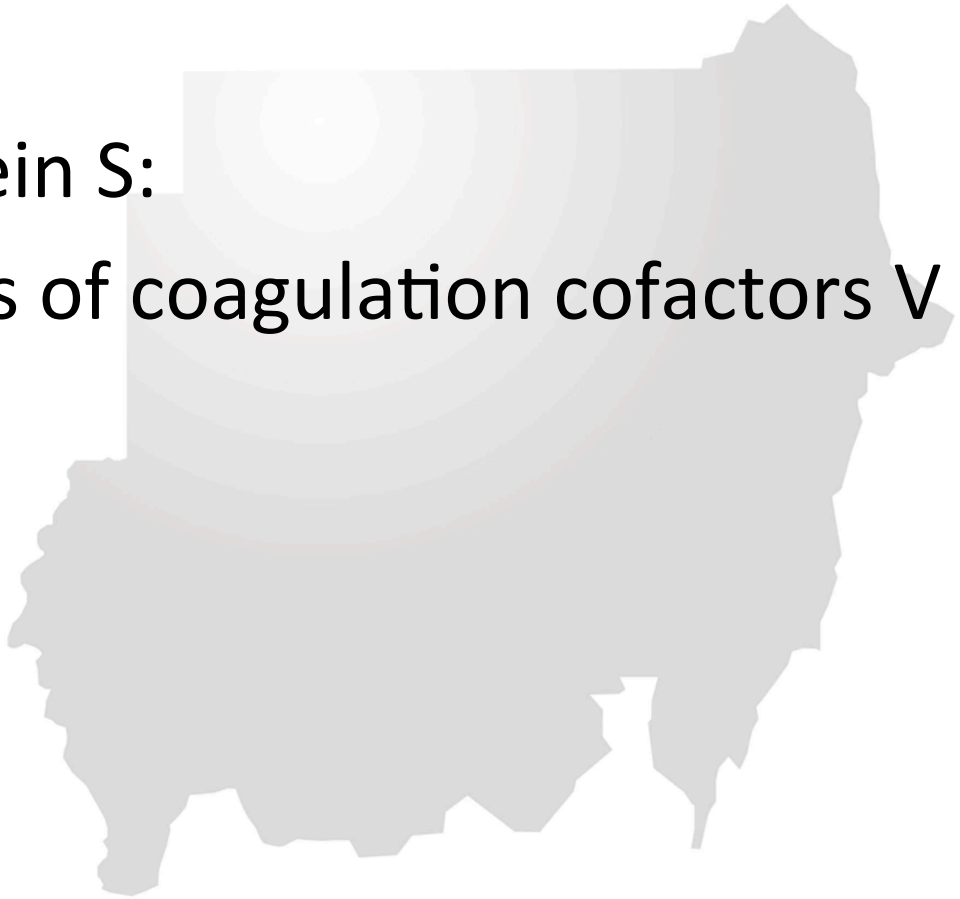
Figure 24.7 The pathway of blood coagulation initiated by tissue factor (TF) on the cell surface. When plasma comes into contact with TF, factor VII binds to TF. The complex of TF and activated VII (VIIa) activates X and IX. TF pathway inhibitor (TFPI) is an important inhibitor of TF/VIIa. The VIIIa–IXa complex greatly amplifies Xa production from X. The generation of thrombin from prothrombin by the action of Xa–Va complex leads to fibrin formation. Thrombin also activates XI (dashed line), V and XIII. Thrombin cleaves VIII from its carrier von Willebrand factor (VWF), greatly increasing the formation of VIIIa–IXa and hence of Xa–Va. Pale green, serine proteases; yellow, cofactors.

- The activity of factors II, VII, IX and X is dependent upon vitamin K (carboxylation of a number of terminal glutamic acid residues).

Coagulation factor inhibitors

- Tissue factor pathway inhibitor (TFPI):
- It inhibits Xa , VIIa and tissue factor by forming the quaternary complex.
- Antithrombin:
- It inactivates thrombin predominantly, then FXa and to some extent FIXa, FXIa, FXIIIa and kallikrein.
- Heparin potentiates its action markedly.

- Protein C and protein S:
- These are inhibitors of coagulation cofactors V and VIII.



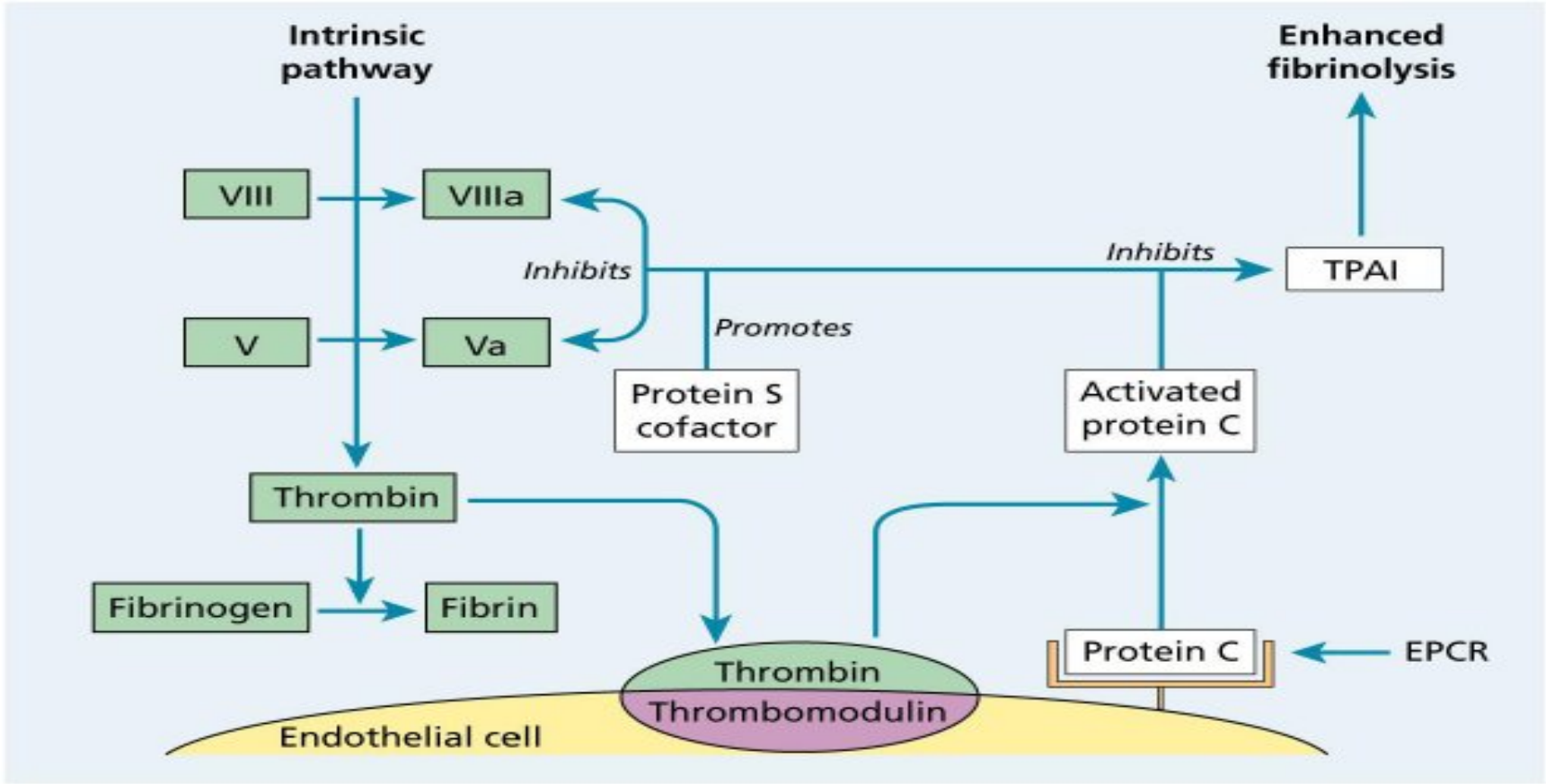


Figure 24.11 Activation and action of protein C by thrombin which has bound to thrombomodulin on the endothelial cell surface. Protein S is a cofactor that facilitates binding of activated protein C to the platelet surface. The inactivation of factors Va and VIIIa results in the inhibition of blood coagulation. The inactivation of tissue plasminogen activator inhibitor (TPAI) enhances fibrinolysis. EPCR, endothelial protein C receptor.

Fibrinolysis

- A normal haemostatic response to vascular injury.
- Plasminogen is a β-globulin proenzyme found in blood and tissue fluid.
- It is converted to the serine protease plasmin by activators either from the vessel wall (intrinsic activation: factor XIIa, kallikrein) or from the tissues (extrinsic activation: tPA, urokinase)

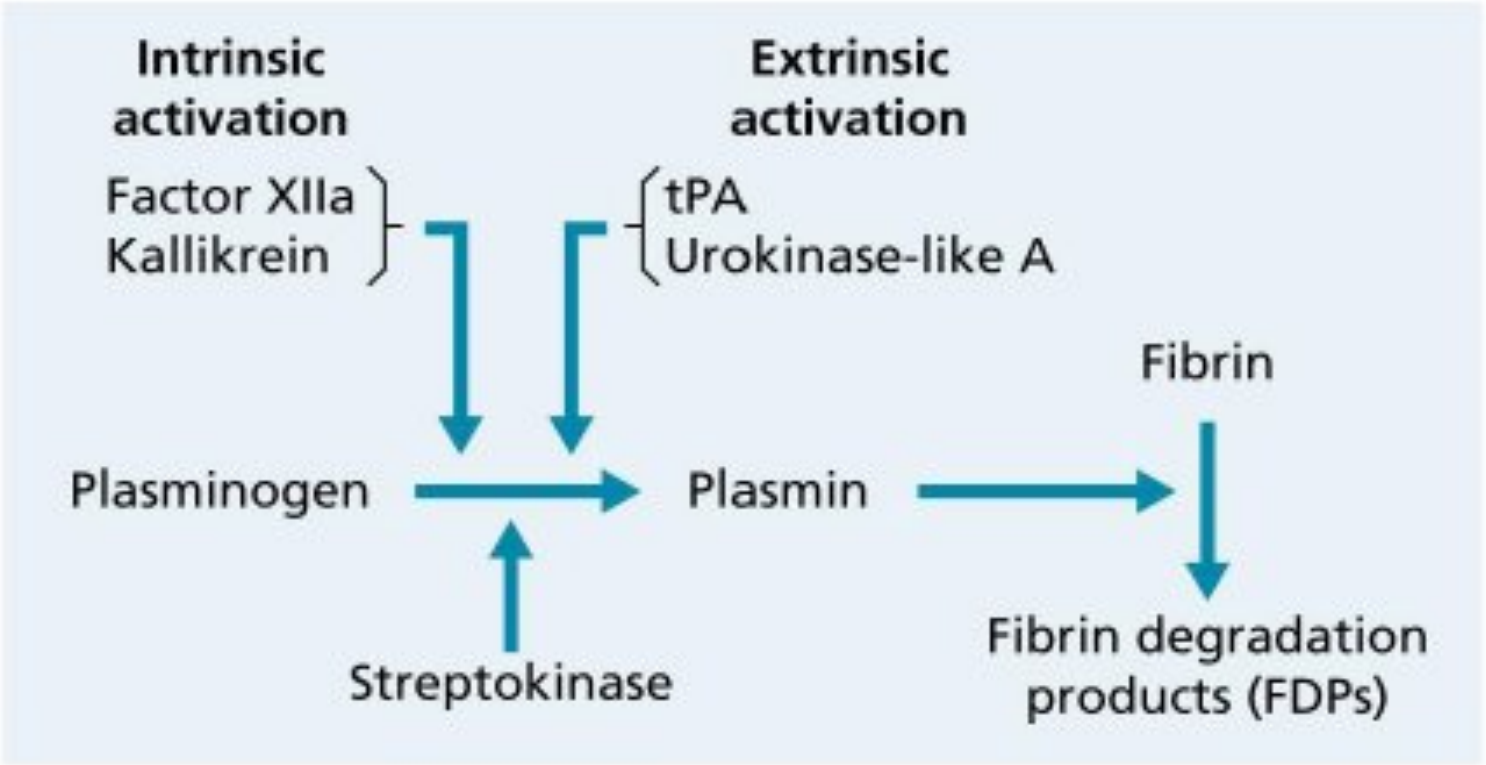


Figure 24.12 The fibrinolytic system. tPA, tissue plasminogen activator.

- The split products of fibrinolysis are competitive inhibitors of thrombin and fibrin polymerization.
- α_2*antiplasmin* inhibits any local free plasmin.
- Plasmin is capable of digesting fibrinogen, fibrin, factors V.

inhibitors of fibrinolysis

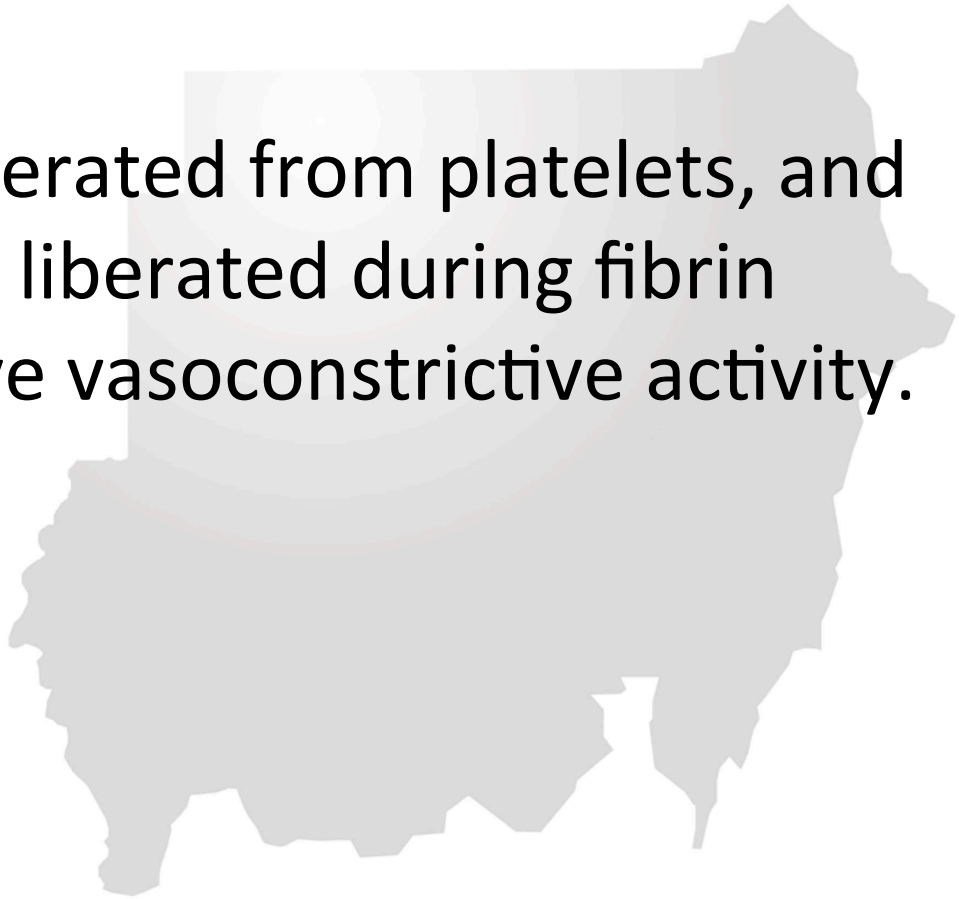
- Tissue plasminogen activator is inactivated by plasminogen activator inhibitor (PAI).
- PAI - 1 is a fast - acting serpin inhibitor of tPA, uPA and, to a small extent, plasmin.
- PAI - 2 is mainly produced by the placenta.

- Fiprenolysis is also inhibited by Thrombin-activated fibrinolytic inhibitor.
- It removes the binding sites of fibrin for PLG and tPA → reducing PLG activation → down regulation of fibrinolysis.

Haemostatic response

- **Vasoconstriction:**
- Immediate vasoconstriction of the injured vessel and reflex constriction of adjacent small arteries and arterioles → initial slowing of blood flow to the area of injury.
- The reduced blood flow allows contact activation of platelets and coagulation factors.

- ThromboxaneA_2 liberated from platelets, and the fibrinopeptides liberated during fibrin formation, also have vasoconstrictive activity.



- **Platelet reactions and primary haemostatic plug formation:**
- Platelets adhere to the exposed connective tissue, potentiated by VWF.
- Collagen exposure and thrombin production cause the adherent platelets to release their granule contents → more platelet activation, prostaglandin and thromboxaneA_2 synthesis.

- Released ADP causes platelets to swell and aggregate.
- Additional platelets from the circulating blood are drawn to the area of injury → the growth of the haemostatic plug which soon covers the exposed connective tissue.

- Prostacyclin, produced by endothelial and smooth muscle cells in the vessel wall adjacent to the area of damage, is important in limiting the extent of the initial platelet plug.

- **Stabilization of the platelet plug by fibrin:**
- Thrombin generated at the injury site converts soluble plasma fibrinogen into fibrin and potentiates platelet aggregation and secretion.
- The fibrin component of the haemostatic plug increases as the fused platelets autolyse.

- After a few hours the entire haemostatic plug is transformed into a solid mass of cross-linked fibrin.
- Because of incorporation of plasminogen and tPA , this plug begins to autodigest during the same time frame.


THANK YOU