Haemostasis

PHYSIOLOGY

DR HUNG DIEP

Introduction

- Haemostasis is the process of forming clots, usually in the context of cessation of bleeding
- Involves 3 major components:
  - **Endothelium**
  - **Platelets**
  - **Coagulation Cascade**
- General process follows:
  - **1. Vasoconstriction**
  - **2. Primary Haemostasis**
  - **3. Secondary Haemostasis**
  - **4. Antithrombotic Counter-Regulation**

Vasoconstriction

- Vasoconstriction by vascular smooth muscle cells is the **first response** to injury (endothelial damage) by blood vessels, predominantly arterioles
- Occurs mostly due to reflex neurogenic mechanisms via vascular endothelium with some contribution by local factors, such as endothelin
- Results in reduced blood flow and limits blood loss
- Platelet granules also promote some degree of vasoconstriction

Primary Haemostasis

- Initiated by endothelial damage, which leads to exposure of subendothelial collagen
- Occurs in 3 steps:
  - **1. Adhesion**
  - **2. Platelet Aggregation**
  - **3. Platelet Activation**
- Platelets bind directly to collagen specific g1a/2b receptors
  - This adhesion is strengthened by vWF and glycoprotein VI, which further activates the platelets
- Activated platelets released stored granules, which leads to a cascade that activates other platelets
  - Granules include ADP, serotonin, platelet activating factor, vWF, thromboxane A2
- Intracellular Ca^{2+} levels increase and leads to processes that increase platelet affinity for fibrinogen binding, which forms cross-links with the glycoprotein 2b/3a complex and completes the primary haemostatic plug

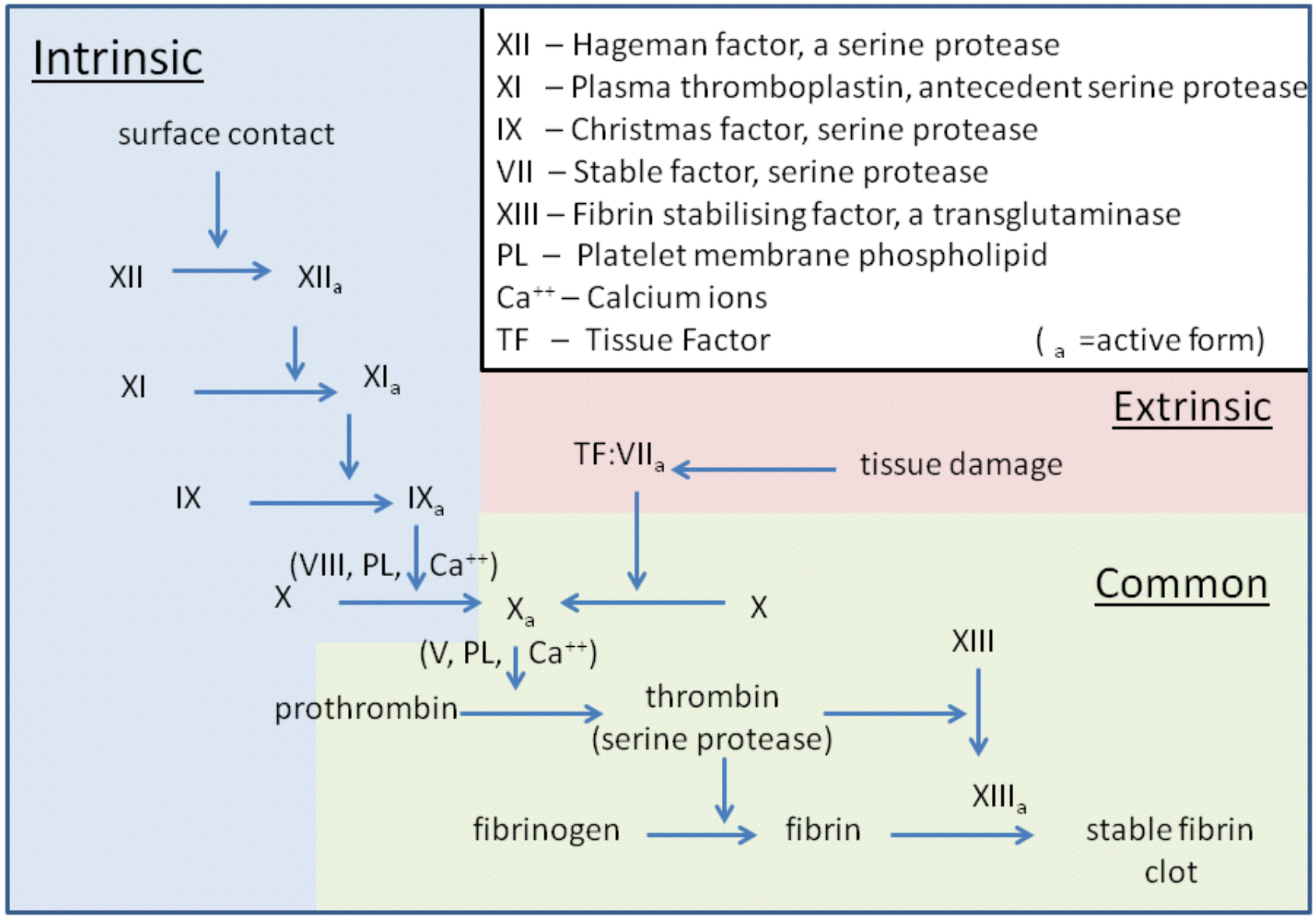
Secondary Haemostasis

- Involves prothrombotic processes to form insoluble, cross-linked fibrin by activated coagulation factors
- Fibrin stabilises the primary platelet plug, to improve its strength in order to cease blood loss
- Processes include:
  - Coagulation Cascade
    - Tissue Factor Pathway (Intrinsic)
    - Contact Activation Pathway (Extrinsic)
  - Co-Factors

Coagulation Cascade

- Involves two pathways that lead to a third common pathway that ultimately activates thrombin to convert fibrinogen to fibrin
  - Contact activation pathway (intrinsic)
  - Tissue factor pathway (extrinsic)
- Tissue factor pathway is the more physiologically important pathway

The three pathways that makeup the classical blood coagulation pathway



Contact Activation Pathway

- Less physiologically important pathway
- Begins with the formation of the primary complex on collagen by high molecular weight kininogen (HMWK), prekallikrein and F XII
- Prekallikrein is converted to kallikrein and F XII becomes F XIIa
- F XIIa converts F XI into XIa, which then converts F X to F Xa and activates F IX to F IXa
- F IXa combines with its cofactor F VIIIa to form a tenase complex that propagates the conversion of F X to F Xa

Tissue Factor Pathway

- Main role is to generate a thrombin burst to facilitate fibrinogen activation
- Endothelial injury results in exposure of tissue factor
- F VII comes into contact with tissue factor and becomes activated (F VIIa)
- F VIIa activates IX and X
  - F Xa then leads to activation of prothrombin (F II) to thrombin (F IIa)
- Activation of F X to form Xa by the tissue factor-F VIIa complex is inhibited by tissue factor pathway inhibitor
- F Xa and F Va form prothrombinase, which converts prothrombin into thrombin

Co-Factors

- Calcium and phospholipid
  - Required for the tenase and prothrombinase complexes to function
- Vitamin K
  - Adds a carboxyl group to glutamic residues on F II, F VII, F IX and F X, and Protein C, S and Z
  - In addition, vitamin K is oxidised during the process, thus inactivating it
    - The enzyme Vitamin K Epoxide Reductase reduces Vitamin K back to its active form
    - Important mechanism in the activity of Warfarin

Antithrombotic Counter-Regulation

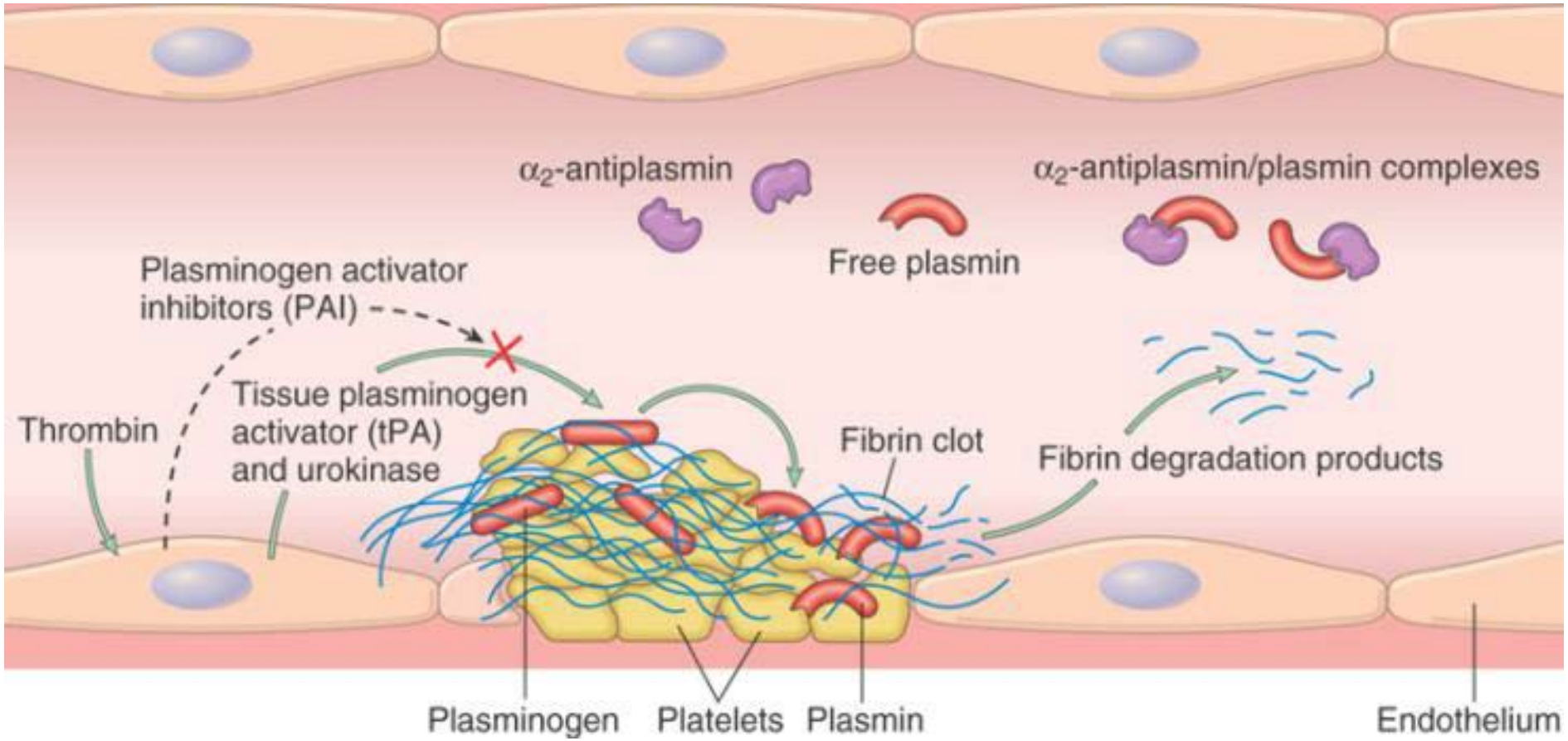
Protein C & S	Major physiological anticoagulant Vitamin K dependent Activated by thrombin and thrombomodulin Degrades F Va and F VIIa, and prevents the formation of further thrombosis
Antithrombin	Serine protease inhibitor – thrombin, F IXa, F Xa, F XIa and F XIIa Constantly active, but its activity is increased by the presence of heparin sulphate
Tissue Factor Pathway Inhibitor	Limits the action of tissue factor Inhibits excessive tissue factor-mediated activation of FVII and F X

Fibrinolysis

- Primary fibrinolysis is a normal body process
  - Activation of coagulation cascade also activates fibrinolytic cascade that moderates the size of the eventual clot
- Secondary fibrinolysis occurs due to medication or medical disorders
- Occurs via activity of plasmin, which breaks down fibrin and interferes with its polymerisation
  - Fibrin split products (including D-dimers) can act as weak anticoagulants
  - Remaining products are cleared by other proteases and excreted in the kidney and liver

Fibrinolysis

- Plasminogen is formed within the liver, circulating within the blood
- Plasminogen cannot cleave fibrin in its native form, but still has affinity for it and is incorporated into the clot when it is formed
- t-PA (tissue plasminogen activator) and urokinase are the agents that convert plasminogen to the active plasmin
- t-PA is released into the blood by damaged endothelium
  - Released slowly so that the clot is broken down over many days
- To prevent excess plasmin activation, free plasmin is rapidly inactivated by α_2-plasmin inhibitor


α2-antiplasmin
α2-antiplasmin/plasmin complexes
Free plasmin
Plasminogen activator
inhibitors (PAI)
Tissue plasminogen
activator (tPA)
and urokinase
Thrombin
Fibrin clot
Fibrin degradation products
Plasminogen
Platelets
Plasmin
Endothelium

Coagulation Studies

- Activated Partial Thromboplastin Time (aPTT) is a measure of the Contact Activation Pathway (intrinsic)
  - Measures Factors I, II, V, VIII, IX, X, XI, XII
- Prothrombin Time (PT) measures the speed of clotting via the Tissue Factor Pathway (extrinsic)
  - Measures Fibrinogen, Prothrombin, and Factors V, VII and X

$$International\ Normalised\ Ratio\ (INR) = \left(\frac{Prothrombin\ Time\ (Test)}{Prothrombin\ Time\ (Normal)}\right)^{International\ Sensitivity\ Index}$$

Coagulation Studies

- Mixing studies involving blood plasma being used to distinguish factor deficiencies from factor inhibitors e.g. lupus anticoagulant, specific factor inhibitors
  - Plasma is mixed in a 1:1 ratio with a sample with 100% normal factors
  - Factor deficiencies will detected when the mixing studies result in a level >50% of normal
  - Factor deficiencies will also lead to a normal prothrombin time (correction with mixing studies)
  - Factor inhibitors are noted if there is failure of correction of the prothrombin time despite mixing

Questions