UNC SCHOOL OF MEDICINE

BIOL 342
Hemostasis and Thrombosis
September 16, 2021

Emma Bouck

PhD Student

emma_bouck@med.unc.edu

Hemostasis and Thrombosis Learning Objectives

I: Background

- Blood
- Vessels
- Flow

II: Disorders

- Hemostatic disorders
- Thrombotic disorders

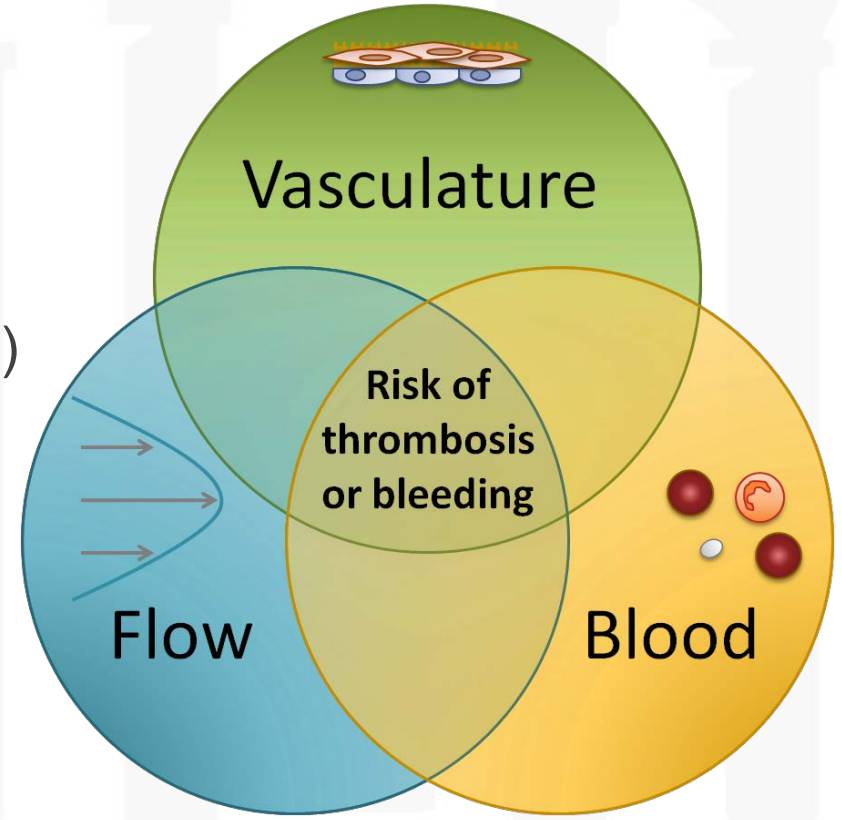
UNC SCHOOL OF MEDICINE

Definitions

- Hemostasis: maintaining blood as a liquid until an injury requires clot formation
- Clotting at inappropriate sites (thrombosis) or migration of clots (embolism) obstructs blood flow to tissues and leads to cell death (infarction).
- Hemorrhage: inability to clot after vascular injury
  - Local bleeding can compromise regional tissue perfusion
  - More extensive hemorrhage can result in hypotension and death

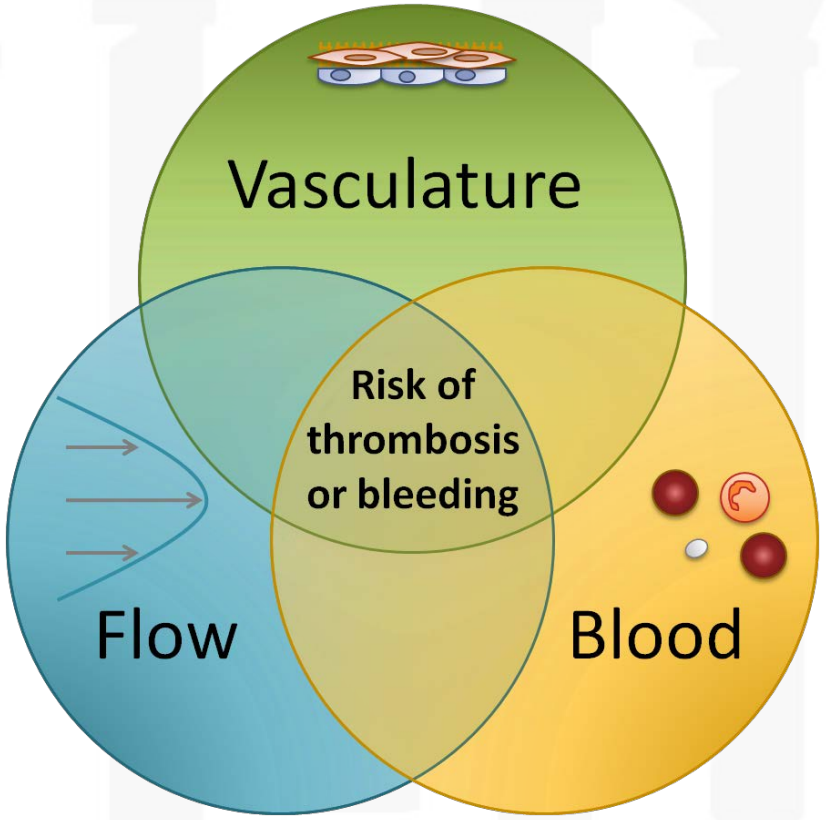
The Components: Virchow's Triad (1856)

- Blood
  - Circulating clotting factors
  - Cells
    - Platelets
    - Leukocytes (white blood cells)
    - Erythrocytes (red blood cells)
- Vasculature
  - Endothelium
  - Subendothelium
- Flow



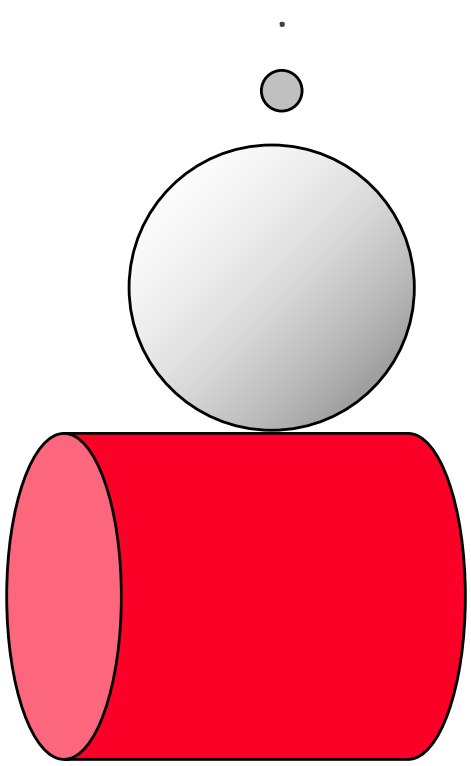
Wolberg et al (2011) Anasth Analg

Blood



Wolberg et al (2011) Anasth Analg

Blood Components to Scale

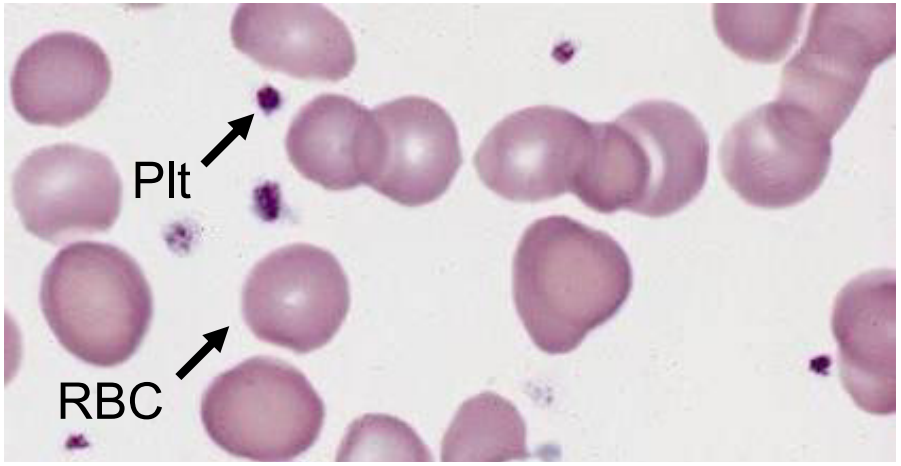


	Diameter	Relative Scale
Fibrinogen	50 nm	1
Platelet	1 μm	20
WBC, RBC	5-9 μm	150
Capillary	5-8 μm	150
Artery	0.02-10 mm	200,000

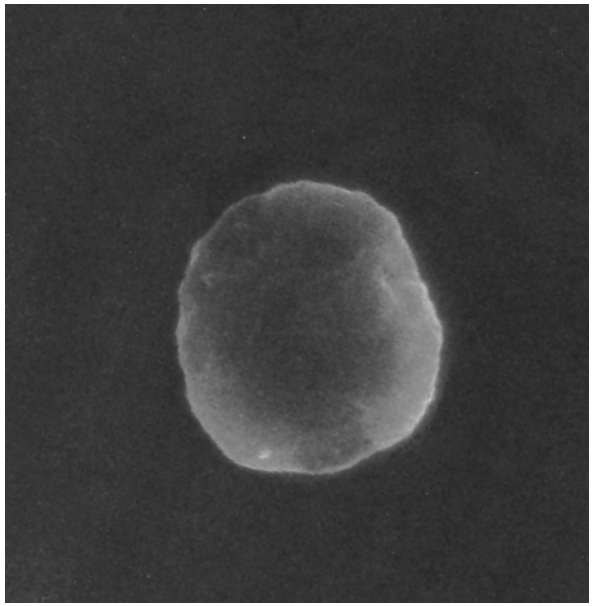
Blood:
Platelets

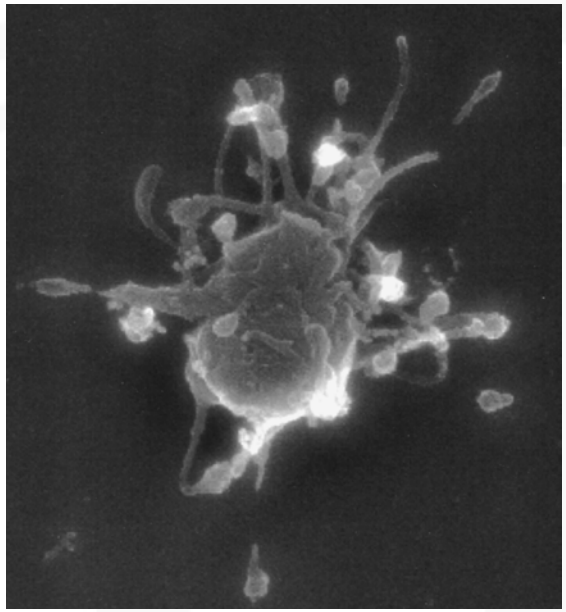
Platelets

Blood smear



- Disc-shaped, anucleate
- Smallest of the blood cells
- Produced in bone marrow from megakaryocytes
- Lifetime ~7-14 days in blood
- Normal = 150-400 x 10^9/L
- Play major roles in hemostasis:
  - Mechanically plug wound
  - Support blood clotting





Resting Platelet

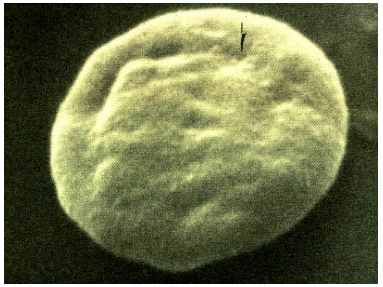
- *Designed to circulate*
- Granule contents sequestered

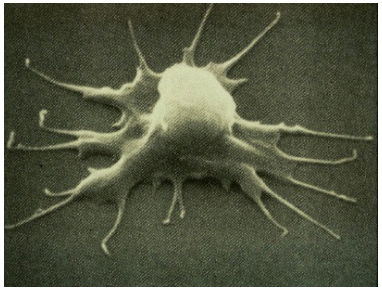
Activated Platelet

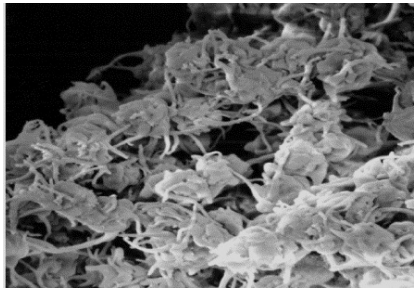
- *Designated to stick & promote coagulation*
- Major reorganization of cytoskeleton
- Receptors are expressed on surface and armed to promote cellular interactions
- Negatively charged phospholipids exposed

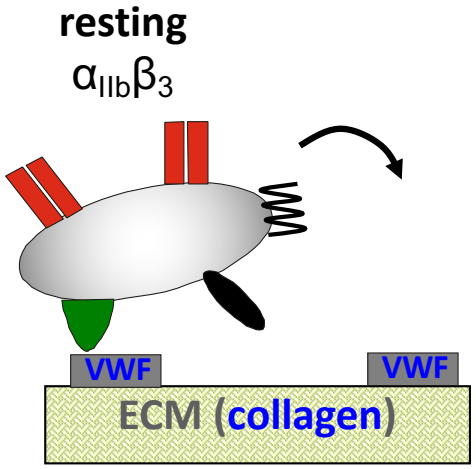
Adapted from Catherine Hayward

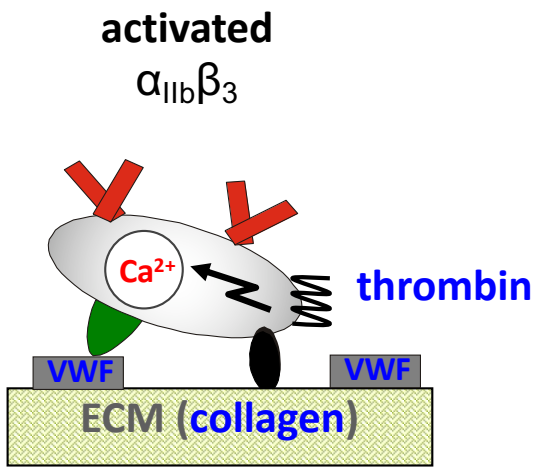
Formation of a Platelet-rich Thrombus

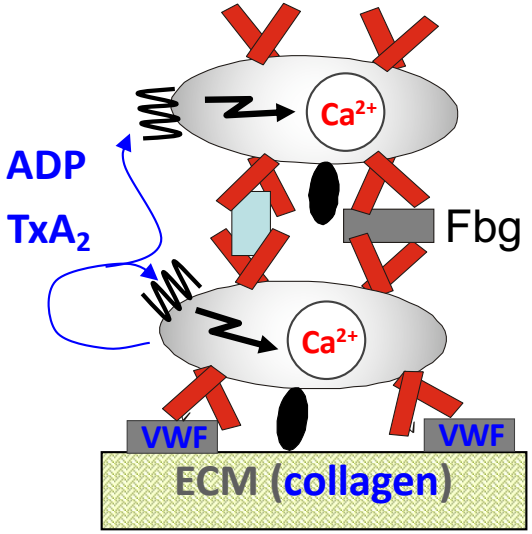












1. initial adhesion (tethering)

2. activation

3. firm adhesion and thrombus growth

Slide from Dr. Bergmeier

Platelet Actions during Clot Formation

- Vascular injury ➔ exposure of subendothelial tissues to blood
- Platelet activation is a 3-step process:
  - » Adhesion: Platelets attracted to material in subendothelial matrix
    - Enhanced by vWF, adheres to platelet membrane glycoprotein Ib (GPIb), fibrinogen binds to platelet integrin GPIIb/IIIa (αIIbβ3)
  - » Secretion: Stimulated platelets secrete materials from alpha and dense granules ("release reaction")
    - platelet surface becomes receptive to coagulation factor binding
  - » Aggregation: Thrombin, thromboxane A_2, ADP stimulate/recruit more platelets
    - GPIIb/IIIa ($\alpha_{IIb}\beta_3$) promotes platelet aggregation, mostly via fibrinogen
- Platelets plug the vascular hole, support fibrin formation, prevent blood loss

Platelets: Vascular First Aid Kits

Alpha-Granules

Platelet membrane proteins:
P-selectin

Adhesive proteins:
vWF, thrombospondin-1,
multimerin, fibrinogen, fibronectin, vitronectin

Coagulation Factors/Proteins:
fibrinogen, factor V, VIII, IX, PAI-1

Chemokines/Modulators of wound healing/angiogenesis:
PF4, TGFβ, PDGF

Soluble proteins:
from plasma (albumin, IgG, etc.)
or made by megakaryocytes

Dense Granules

Substances that activate platelets:
serotonin, ADP, epinephrine

Other nucleotides:
GTP, ATP

Membrane proteins:
CD63, P-selectin, others

Calcium, magnesium, phosphorous,
pyrophosphate, polyphosphate

Lysosomes

Enzymes
CD63

...designed to activate at a moment's notice

Adapted from Catherine Hayward

Smith et al (2006) PNAS 103:903; Smith et al (2008) Blood 112:2810

Blood:
Soluble Coagulation Proteins

Proteins Involved in Coagulation

- Coagulation factors are plasma proteins synthesized primarily in the liver, except:
  - vWF, FVIII (endothelial cells and megakaryocytes)
  - Factor XIII (A-subunit in cells of megakaryocyte origin, B-subunit in liver)
- Designated by Roman numerals in order of discovery

Madeira et al (2009) Blood 113: 5364-5

Blood Coagulation Factors

I	Fibrinogen
II	Prothrombin
III	Tissue thromboplastin (tissue factor)
IV	Calcium
V	Labile Factor, Proaccelerin
VI	------
VII	Stable Factor, Proconvertin
VIII	Antihemophilic factor
IX	Christmas factor
X	Stuart factor
XI	Antihemophilic factor C
XII	Hageman factor
XIII	Fibrin stabilizing factor

Rufus and Ella Stuart in 1975

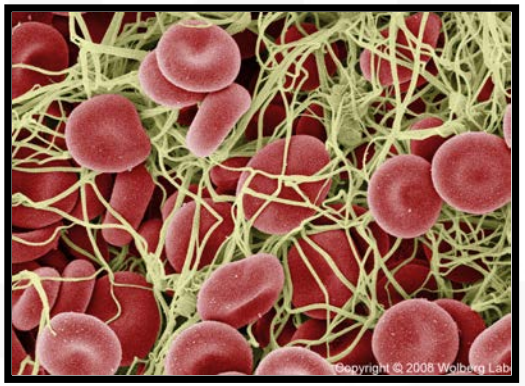
JB Graham (1988) NC Med J 49(6):328-331

Blood Coagulation Factors

- Zymogens/active enzymes:
  - Serine proteases (II, VII, IX, X, XI, XII)
  - Transglutaminase (XIII)
- Cofactors
  - V, VIII, TF, vWF
- Non-protein cofactors
  - Calcium and phospholipids
- Fibrinogen
  - Converted to fibrin by thrombin

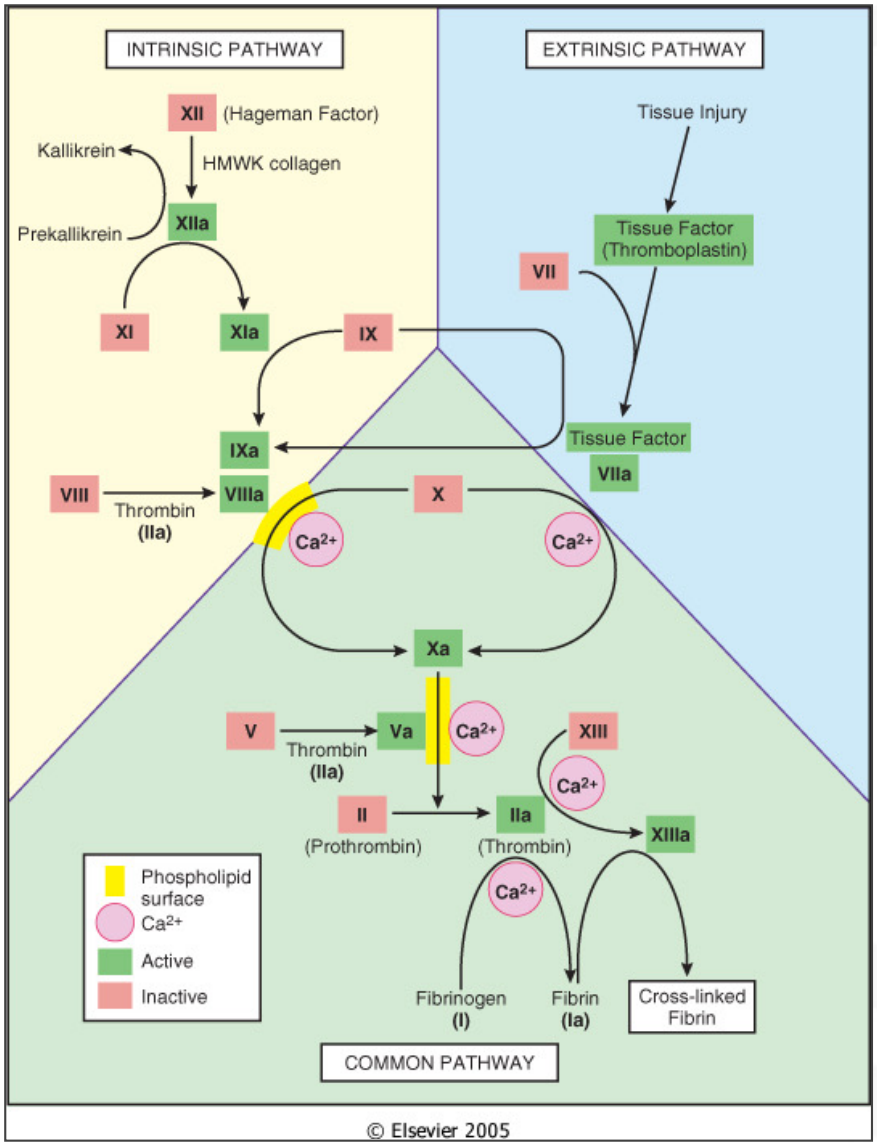
Coagulation Cascade

- "cascade" or "waterfall"
- Historically divided into two pathways:
  - Intrinsic pathway - FXII contacts thrombogenic surface
  - Extrinsic pathway - Blood exposure to tissue factor (TF)
- Thrombin generation is the most important step in clot formation and its subsequent stabilization
- Result: thrombin converts soluble fibrinogen into insoluble, polymerized fibrin



Davie and Ratnoff (1964) Science; Macfarlane (1964) Nature

Historical Coagulation Cascade

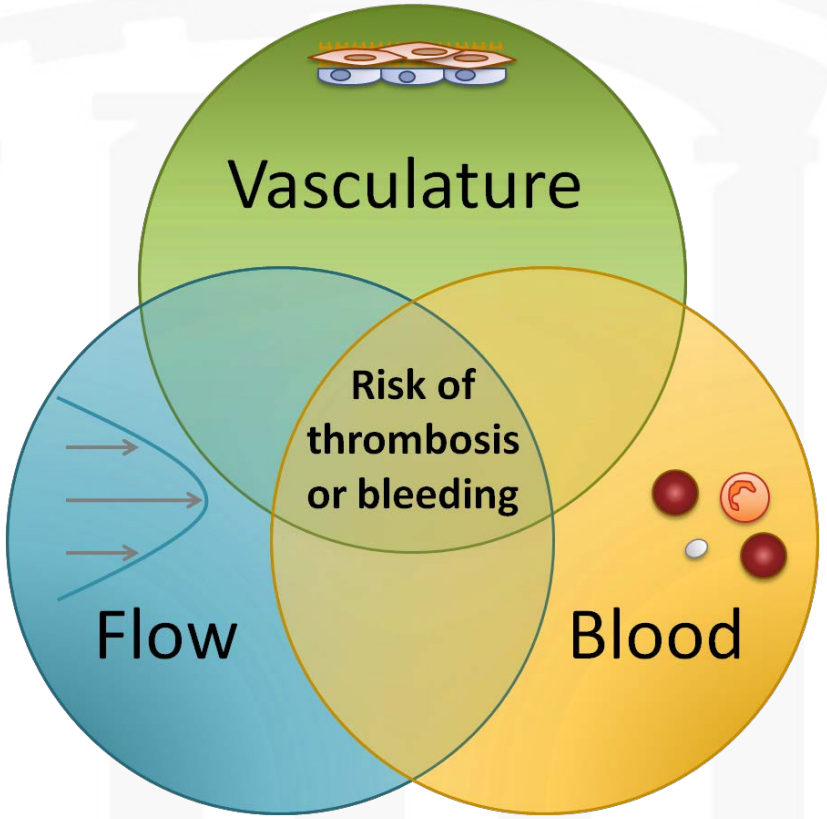


- Series of regulated proteolytic reactions leading to conversion of fibrinogen to fibrin and formation of a stable clot
- Why?
  - Amplification
  - Control

Did you know…

There is enough clotting *potential* in 1 mL of blood to clot all the fibrinogen in the body.

Vasculature



Wolberg et al (2011) Anasth Analg

Vasculature

- Designed for smooth movement of blood from heart to tissues to heart
- Arteries: High flow; Large, elastic/muscular vessels control distribution of blood
- Capillaries: Very low flow; Small (essentially endothelial cells + pericytes); allow exchange of nutrients, O_2
- Veins: Low flow; Return blood to heart; Help control blood volume; Valves prevent backflow

Endothelium - Multiple Functions

Procoagulant Activities

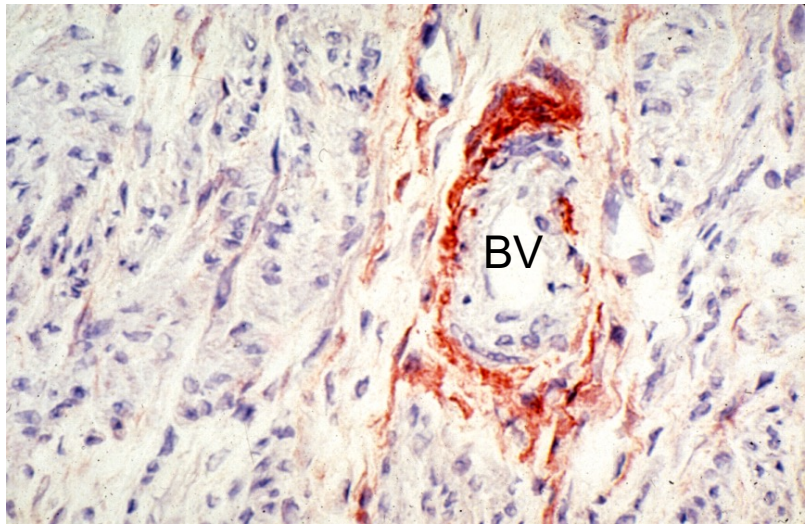
- Pro-platelet:
  - Produces and stores vWF, releases vWF during endothelial activation, which recruits platelets to wound site
- Procoagulant:
  - Triggers clotting via tissue factor expression? (controversial)
- Anti-fibrinolytic:
  - Suppresses fibrinolysis by secreting plasminogen activator inhibitors (PAIs)

Antithrombotic Activities

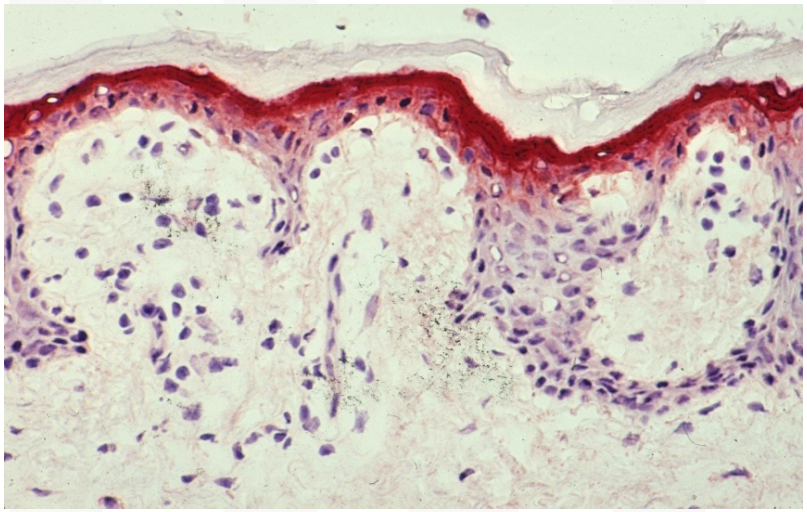
- Anti-platelet:
  - ADPase inhibits platelet aggregation
- Anticoagulant:
  - Inhibits initiation of coagulation (Tissue Factor Pathway Inhibitor)
  - Inhibits coagulation with heparin sulfate proteoglycans that support anticoagulant activity of **antithrombin**
  - Down-regulates coagulation with thrombomodulin that activates **protein C** system
  - Separates blood component from collagen and TF expressing cells
- Fibrinolytic:
  - tPA activates plasminogen to plasmin, which **lyses fibrin**

Envelope Theory – hemostatic TF

Blood Vessel

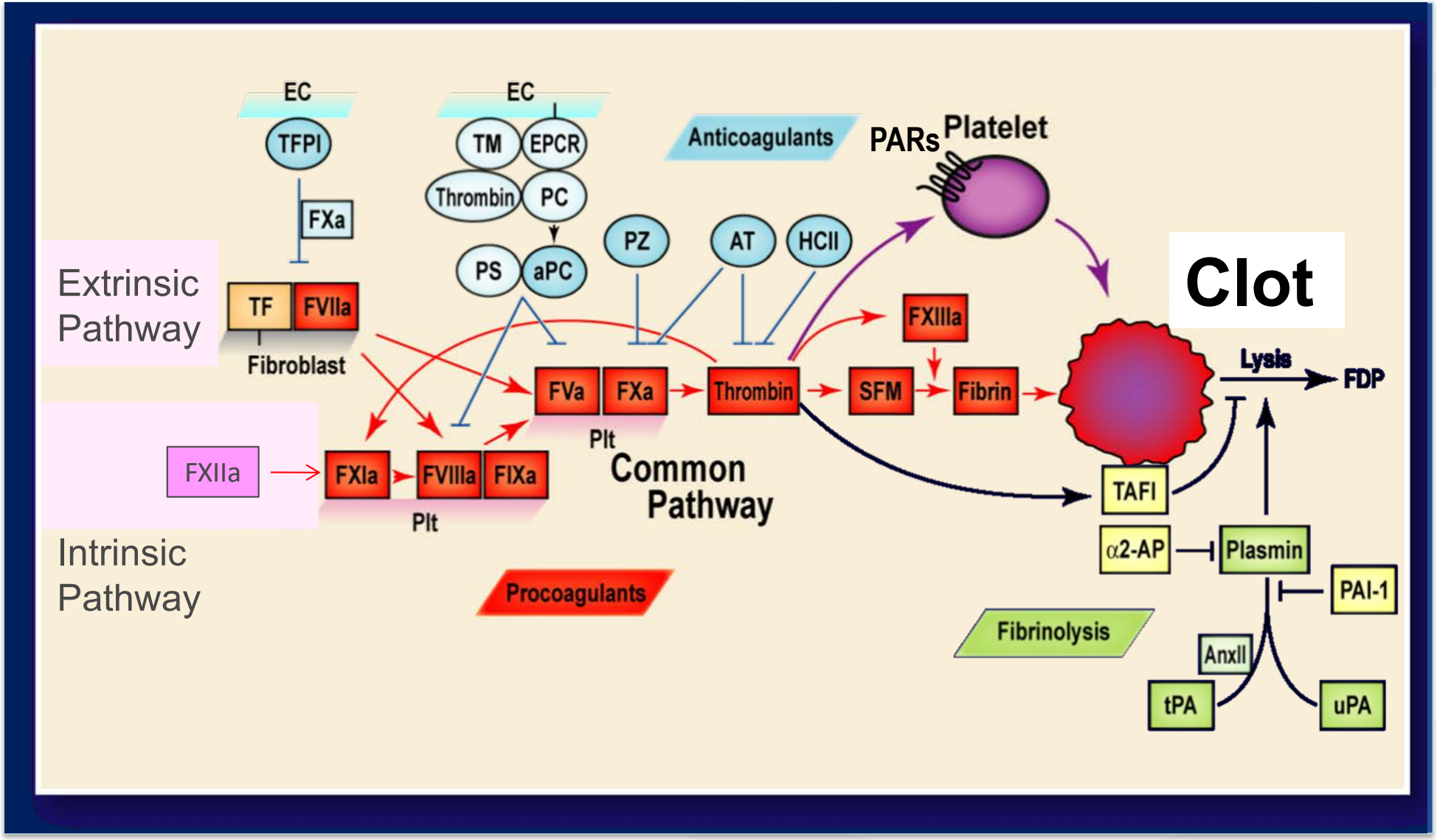


Skin



Drake et al AJP 1989

Formation and Lysis of a Clot

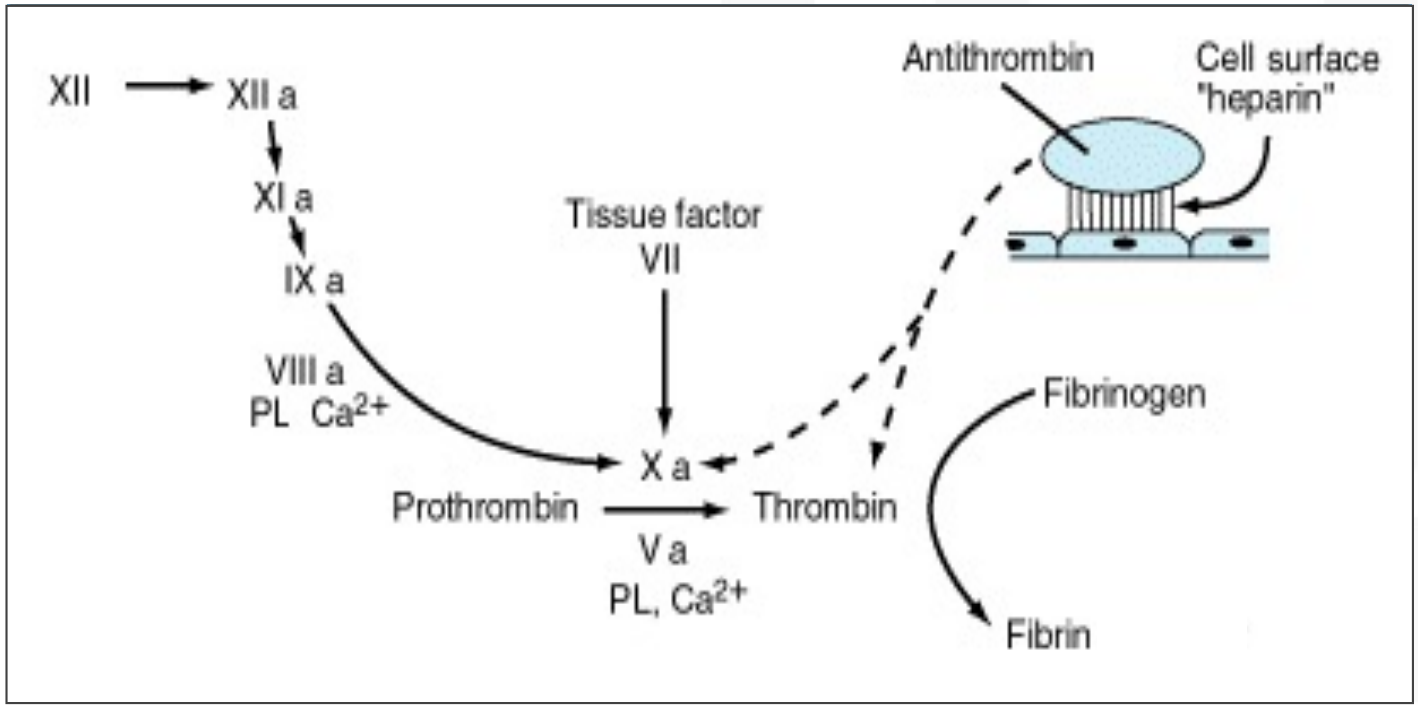


Mackman, *ATVB.* 2005

Endogenous Inhibitors of Blood Coagulation

Antithrombin:

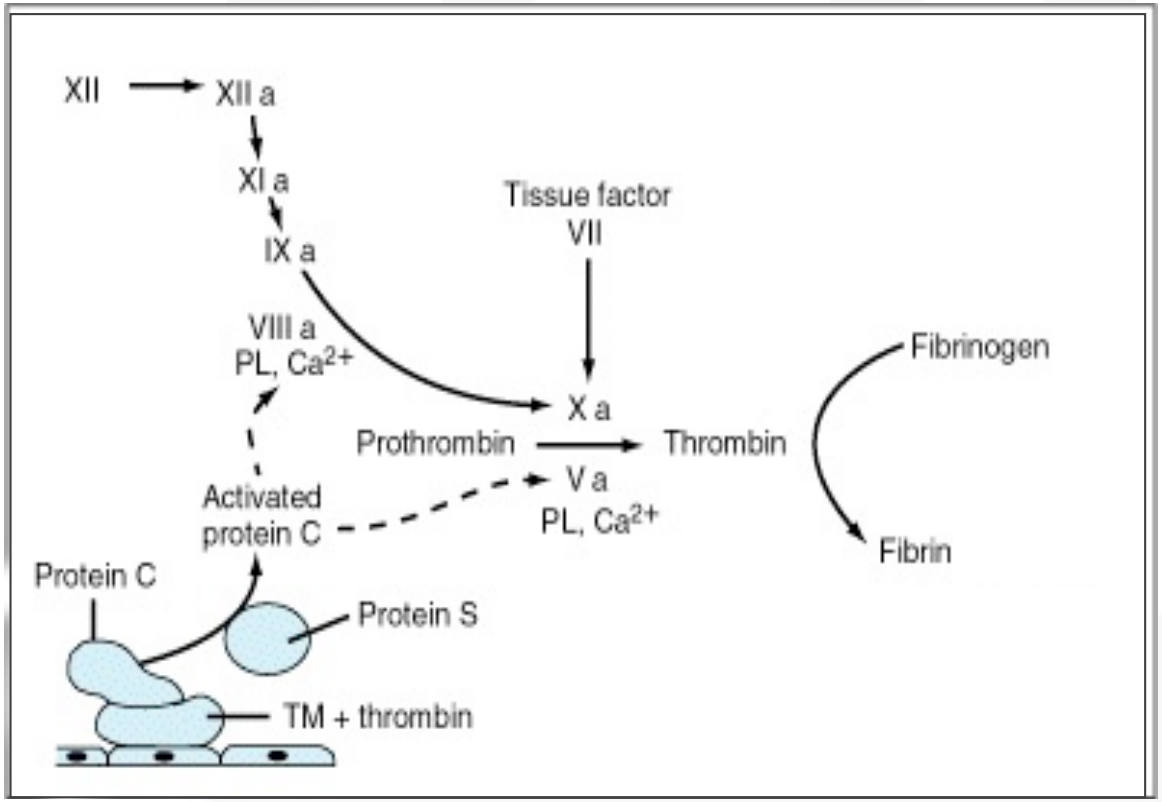
- Inhibits serine protease coagulation factors
- Mainly targets factor Xa and thrombin
- Activity is increased by heparin and heparin-like molecules on the endothelial surface



Endogenous Inhibitors of Blood Coagulation

Protein C System:

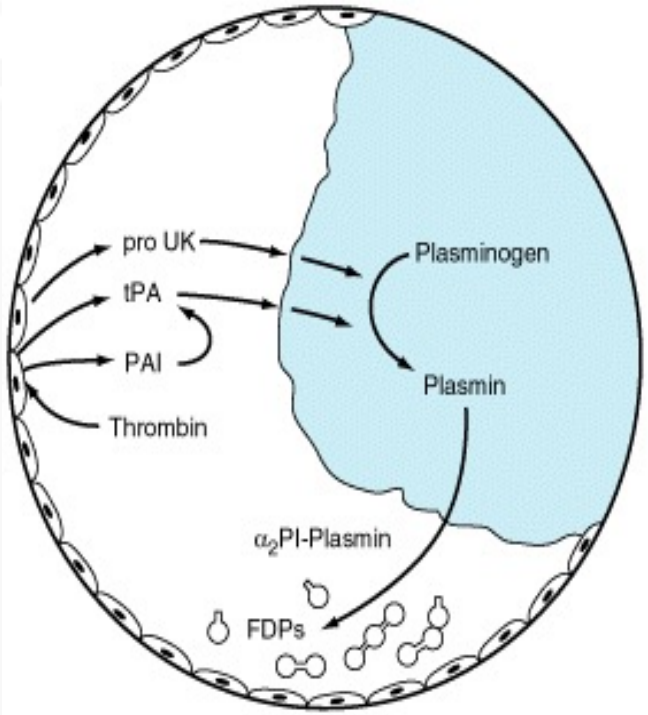
- Thrombin binds to thrombomodulin on endothelial cells
- Thrombin/thrombomodulin complex converts protein C to activated protein C (APC)
- Activated protein C *inactivates* cofactors Va and VIIIa by proteolysis
- APC activity is enhanced by protein S



Endogenous Inhibitors of Blood Coagulation

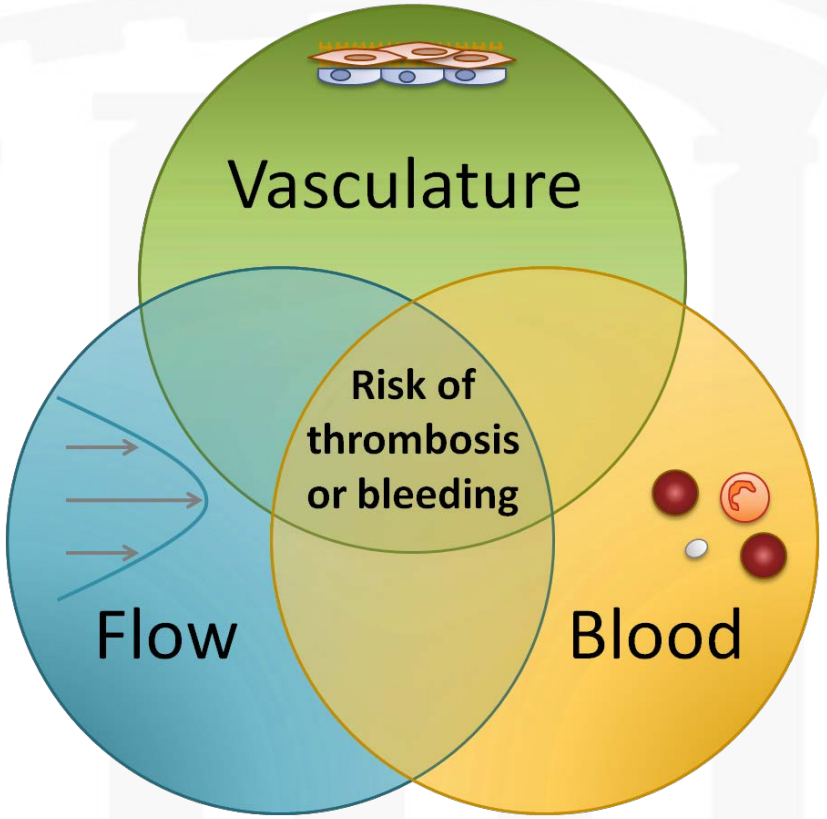
Fibrinolysis:

- Tissue plasminogen activator (tPA) and urokinase (uPA)
  - Produced in endothelial cells and leukocytes
  - Convert plasminogen into plasmin



- Plasmin degrades fibrin into small fragments (D-dimer)
- tPA, uPA are inhibited by plasminogen activator inhibitor-1 (PAI-1)
- Plasmin is inhibited by α2-antiplasmin (α2-PI)

Flow



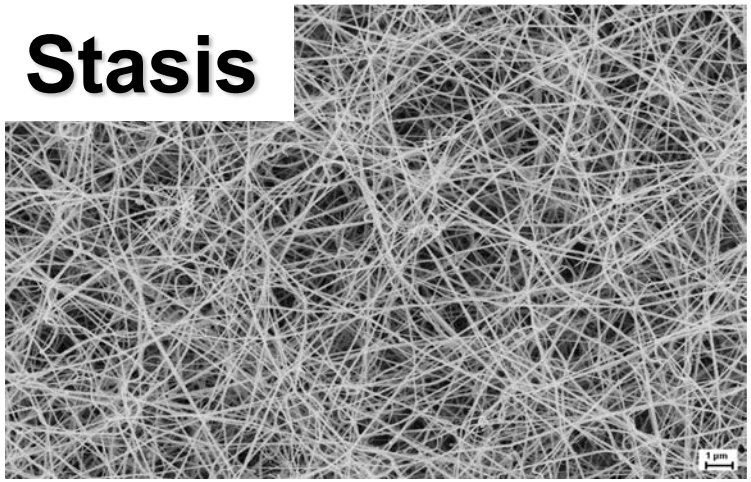
Wolberg et al (2011) Anasth Analg

Flow (Shear) Influences Clotting

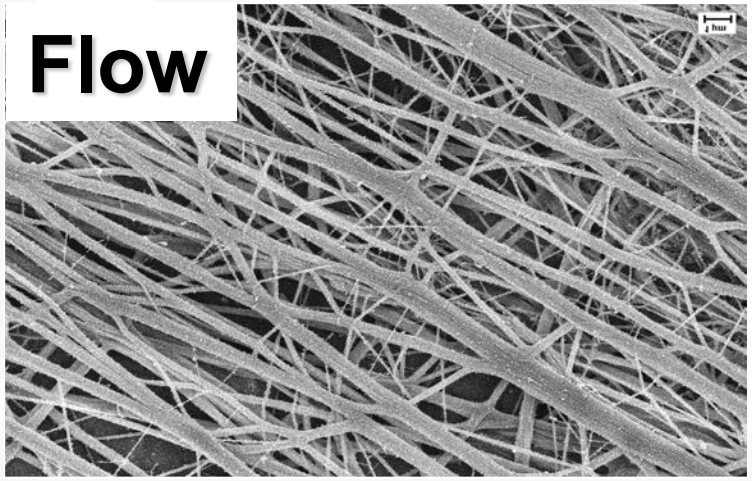
Blood flow

- supplies procoagulants
- promotes cell adhesion
- removes products
- dictates local thrombin concentration
- produces anisotropic fibrin

Stasis



Flow



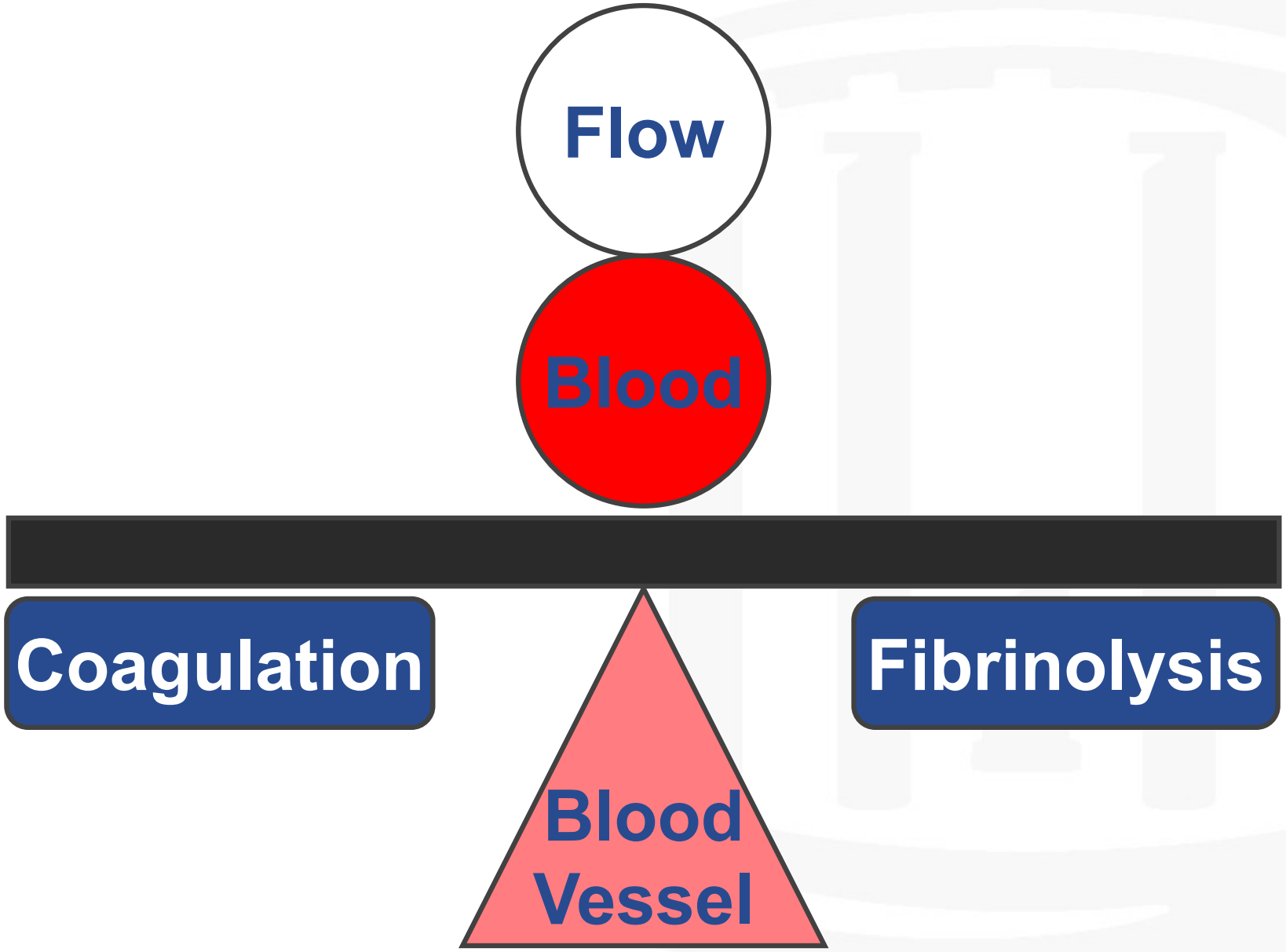
Campbell, Aleman et al (2010) Thromb Haem

Fitting it all together…

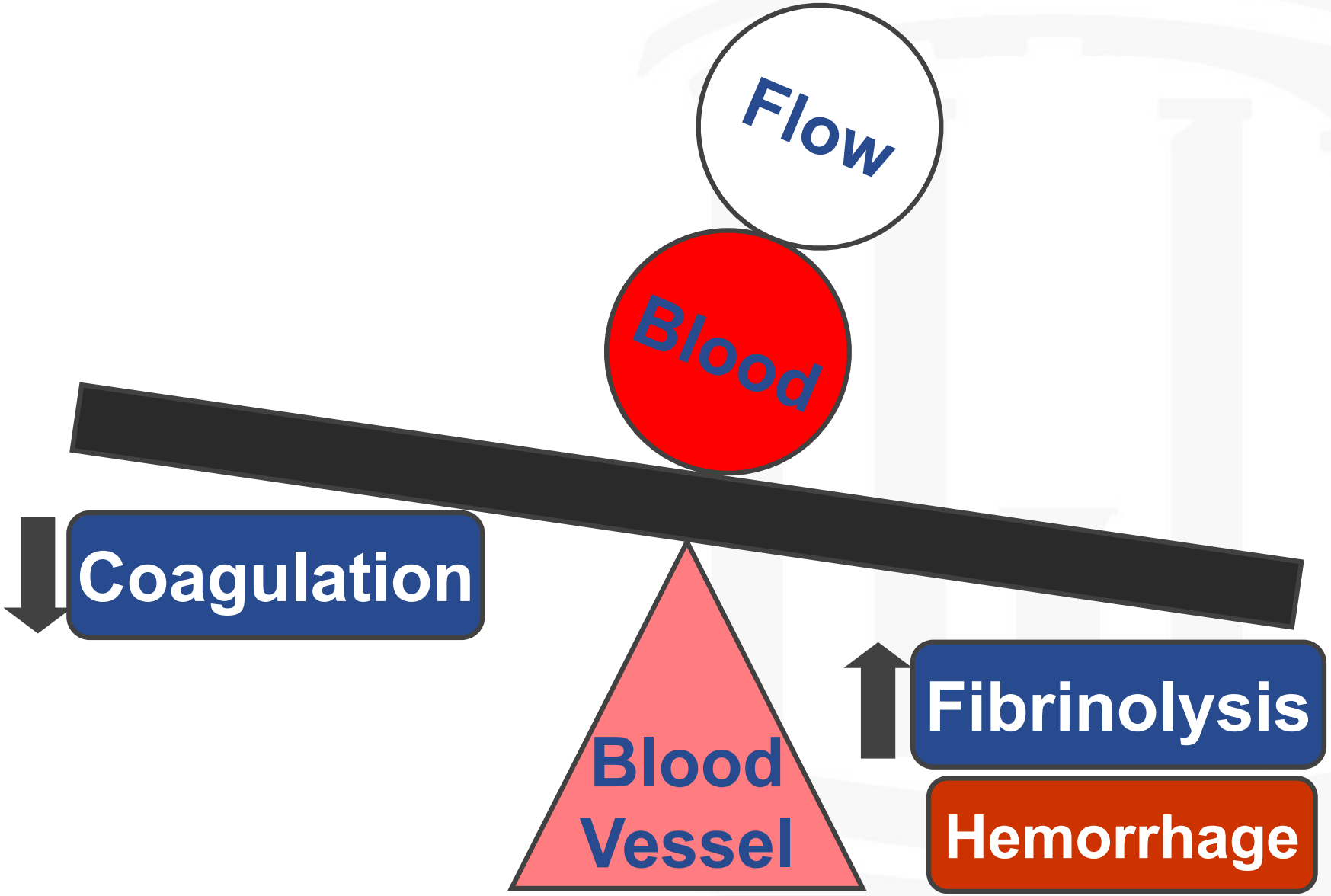
Primary hemostasis: Interactions of vessel wall (endothelial and subendothelial cells) and platelets to form initial hemostatic plug

Secondary hemostasis: Process of blood coagulation involving enzymes and cofactors

"Balance" of Hemostasis

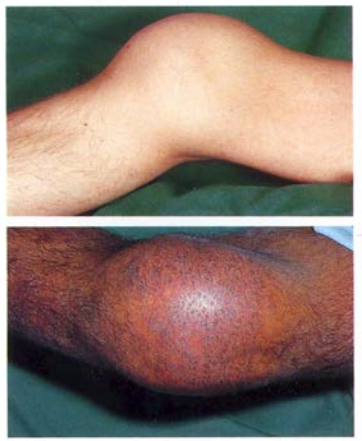


"Balance" of Hemostasis

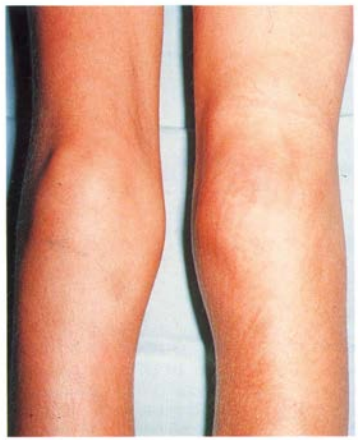


Bleeding Disorders: Hemophilia

- Reduced factor VIII (Hemophilia A) or factor IX (Hemophilia B) or reduced factor XI (Hemophilia C)
- Symptoms: deep muscle and joint hemorrhage, hematomas, post-traumatic bleeding, intracranial bleeding
- Hemarthroses, synovial hypertrophy, erosion of joint cartilage and bone
- Hemorrhage can be severe and life-threatening
- Treatment by clotting factor replacement or bypassing therapies

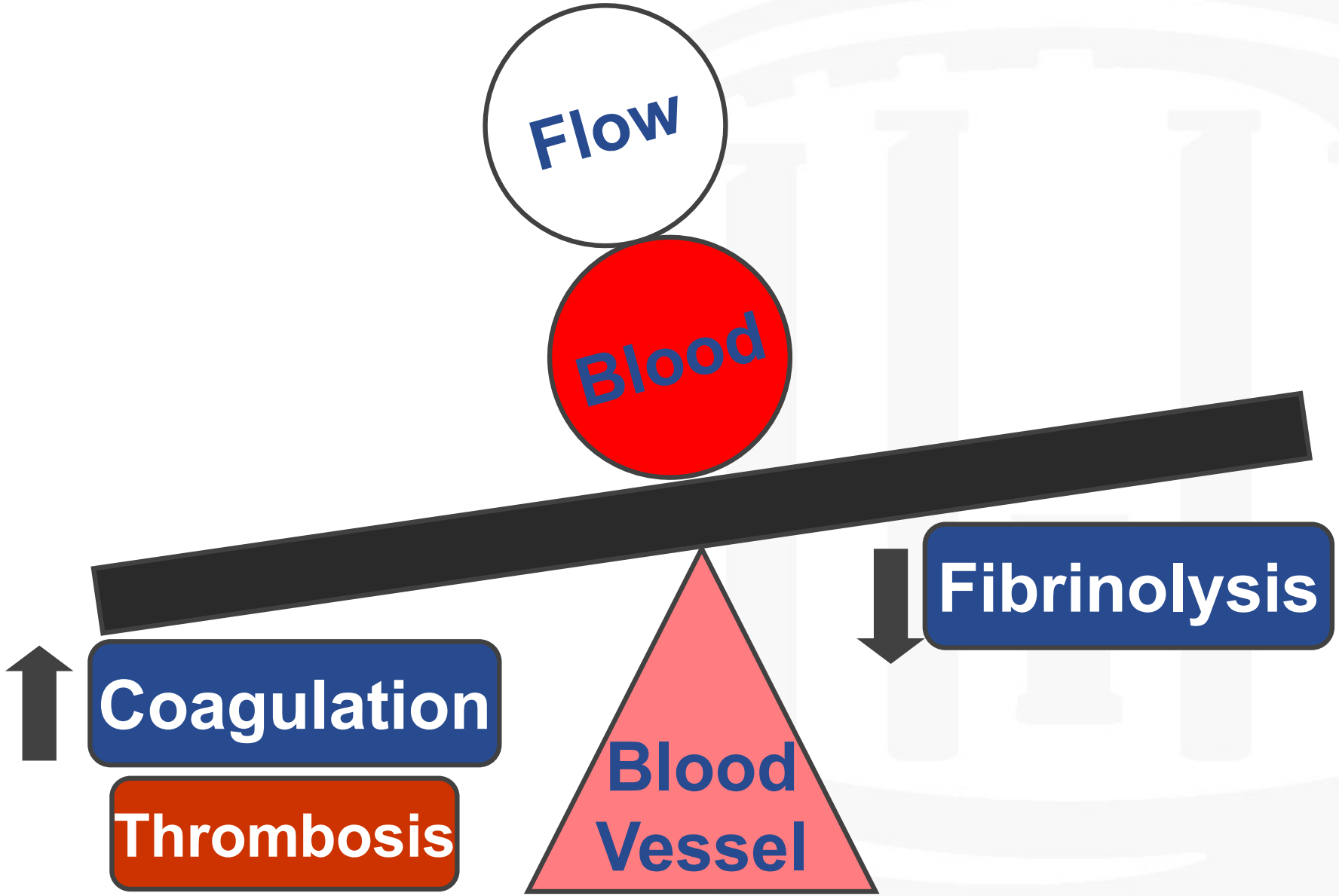


Haemophilia A: gross swelling due to acute haemarthroses of the knee joints.



Haemophilia A: acute haemarthrosis of the left knee joint, with swelling of the suprapatellar area. There is wasting of the quadriceps muscles, particularly on the right.

"Balance" of Hemostasis



Thrombosis

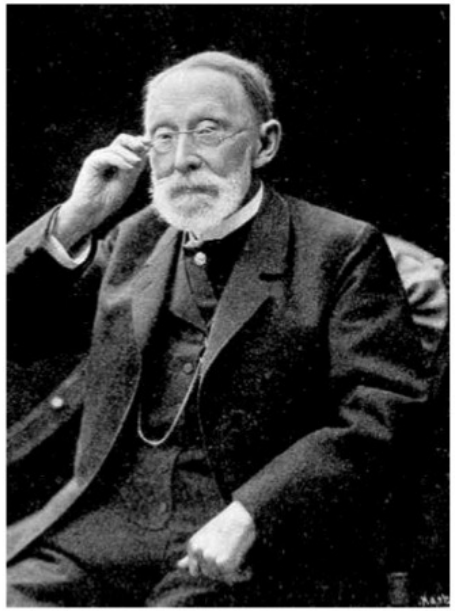
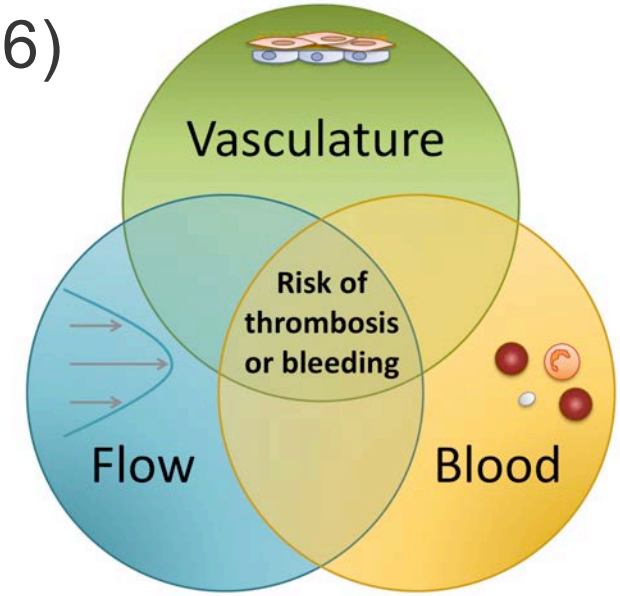


Fig 1. Rudolf Virchow, 1902, the year of his death at the age of 81 (Adapted from: http://commons.wikimedia.org/wiki/Image:Grab_rudolf_Virchow.jpg).

- Thrombosis: pathological process in which clotting is activated inside a blood vessel
- Rudolph Virchow (1856)
- Abnormalities in:
  - Blood composition
  - Vasculature
  - Blood flow

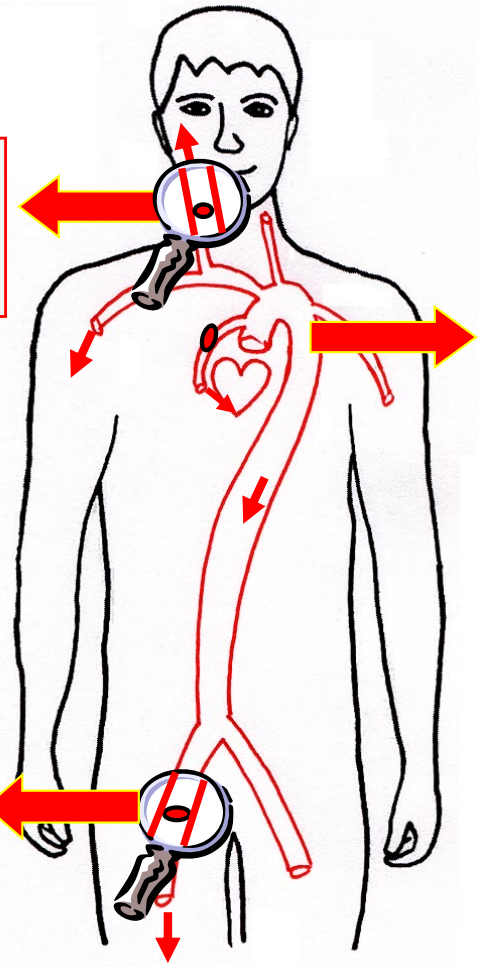


Bagot and Arya (2008) Brit J Haem 143:180-90;
Wolberg et al (2012) Anesth Analges 114:275; Wolberg et al (2015) Nat Rev Dis Pri 1:15006

Arterial thrombosis

Stroke

TIA or ministroke

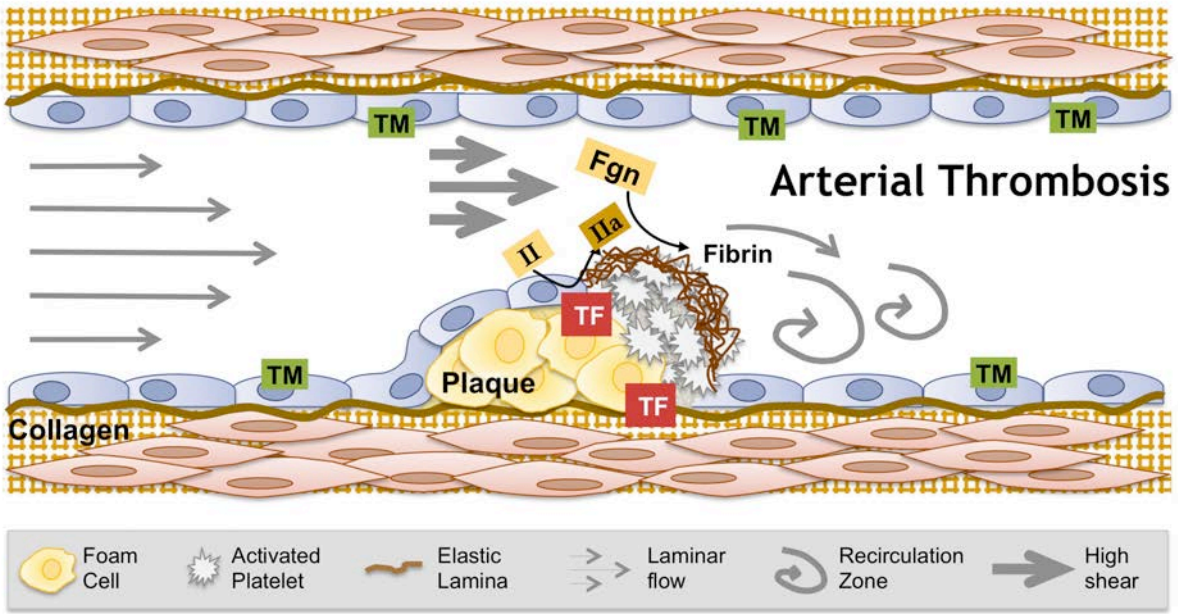


Heart attack

Leg or arm arterial clot (= gangrene):

cold, white (later black), painful

Arterial Thrombosis



Wolberg et al (2012) Anesth Analgesia

- Most common cause of death in Western industrialized countries
- Associated with vascular injury and altered flow, most commonly secondary to atherosclerosis
- "White thrombus" reflects high platelet content

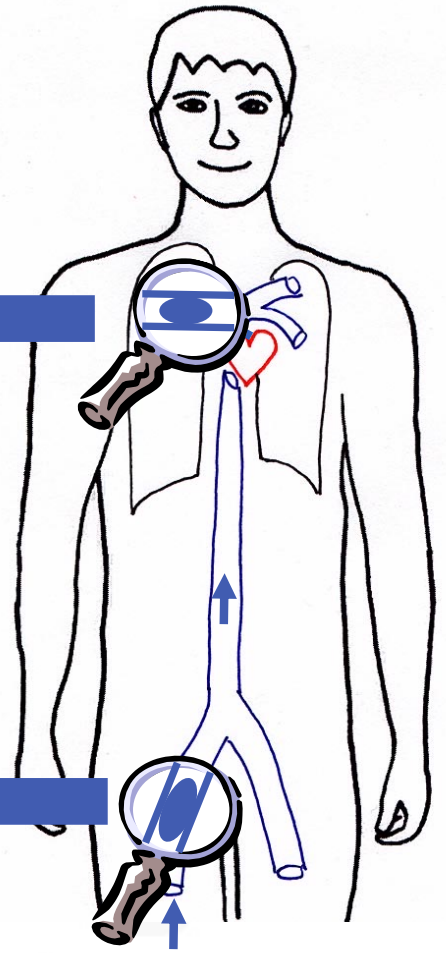
Venous thrombosis

Pulmonary embolism (PE)

- shortness of breath
- chest pain
- cough, bloody phlegm
- fast heart beat

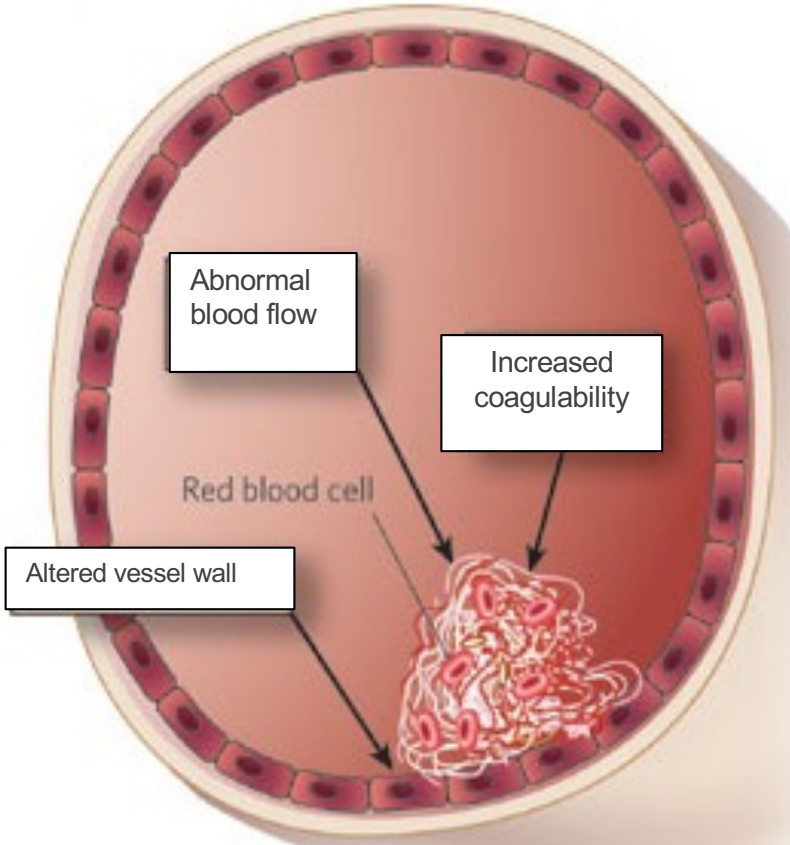
Deep vein thrombosis (DVT)

- swollen
- painful
- warm
- blue-purple



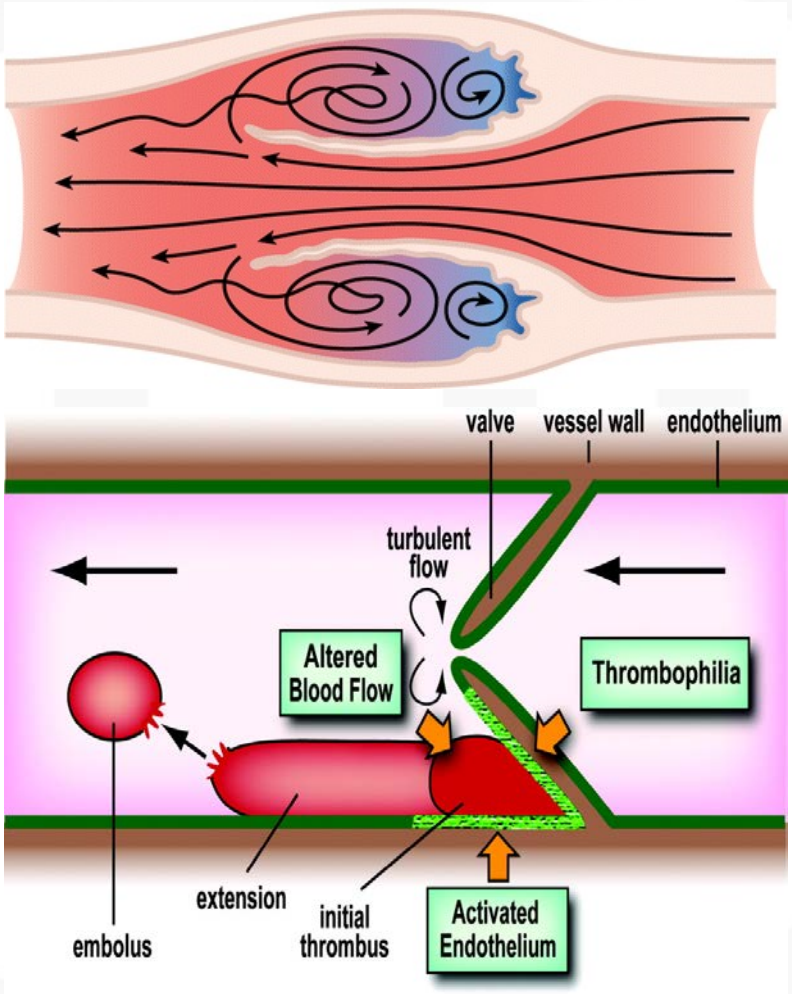
Formation of a Deep Vein Thrombus

Red clot (RBC-rich, fibrin-rich)



Virchow's triad

Mackman Nature 2008

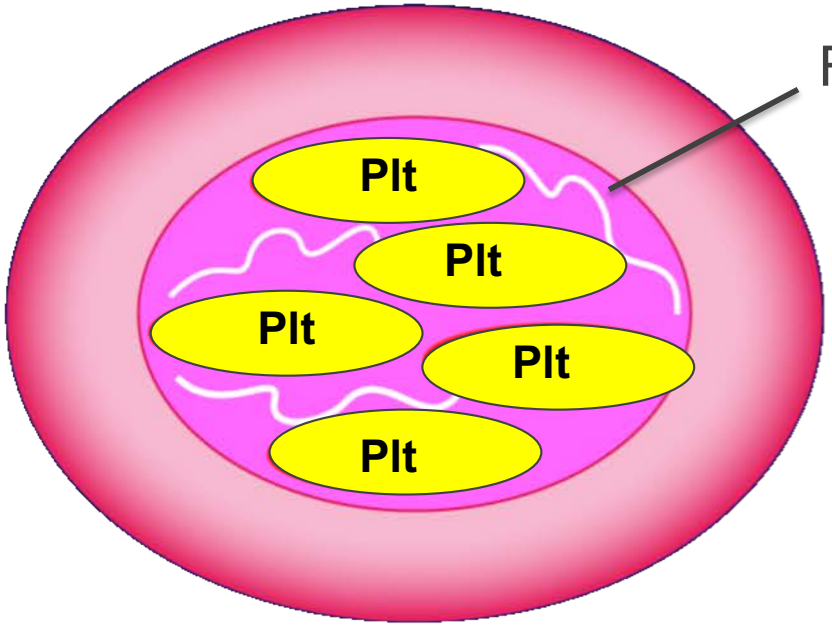


Bovill Ann Rev Physiol 2011; Moll and Mackman ATVB 2008

Composition of Clots in Arteries and Veins

Arterial Thrombosis

(white clot)



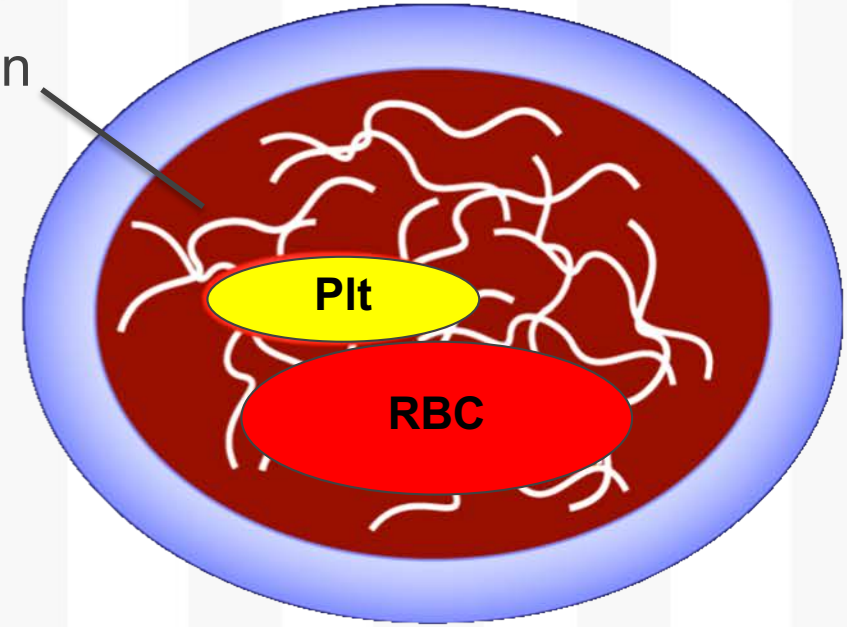
Platelet-rich

Anti-platelet drugs

Anti-coagulant drugs

Venous Thrombosis

(red clot)

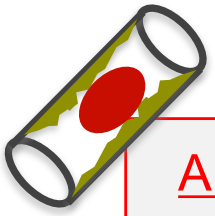


Fibrin-rich

Anti-coagulant drugs

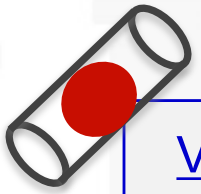
Anti-platelet drugs

Risk factors for thrombosis



Artery Clots

1. Smoking
2. Overweight
3. High cholesterol
4. High blood pressure
5. Diabetes



Vein Clots

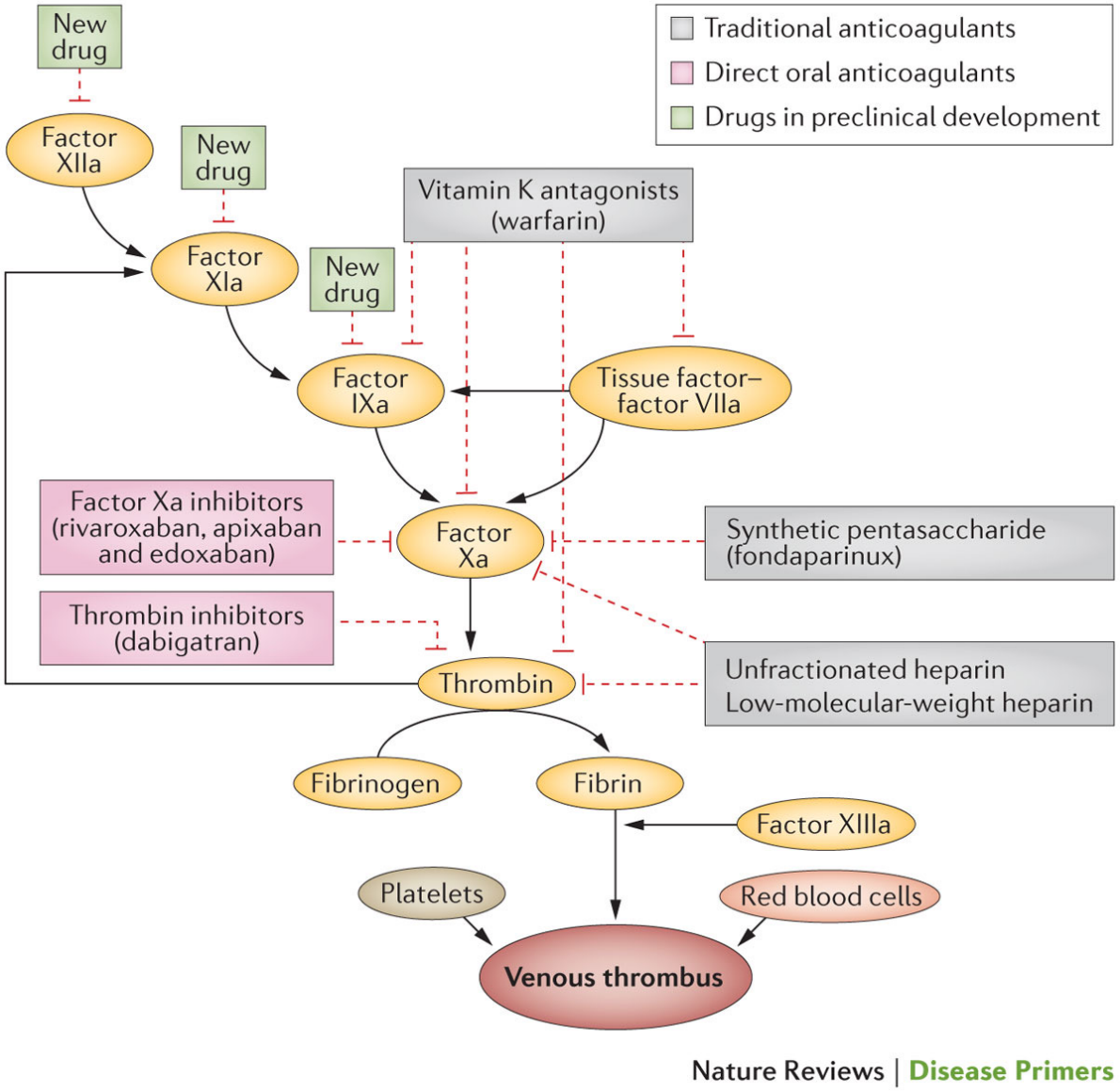
1. Immobility
2. Overweight
3. Surgery, trauma
4. Estrogens, pregnancy
5. Cancer
6. Clotting disorders (= thrombophilia)

Treatment

- Treatment and prevention of thrombosis differs depending on whether it involves arterial or venous circulation

- Fibrinolytic enzymes (e.g. tPA) used to treat acute thrombosis in both arteries and veins
- Anti-platelet drugs (e.g. aspirin) used to prevent arterial thrombosis
- Anticoagulant drugs ("blood thinners", e.g. heparin, warfarin) used to prevent venous thrombosis

Existing & Emerging Anticoagulant Drugs



Wolberg *et al* (2015) Venous thrombosis. *Nat Rev Dis Primers*

UNC SCHOOL OF MEDICINE

Take Home Messages

- Hemostasis is the physiological balance between bleeding and clotting
- Blood coagulation involves contributions from the blood cells and plasma proteins, the vasculature, and the flow of blood in the vessels
  - Each of these is designed to be anticoagulant in the resting state and activation induces a procoagulant state
- Arterial thrombosis relies on platelets whereas venous thrombosis relies on coagulation proteins