Hemostasis: cessation of blood loss from a damaged vessel
→ depends on vessel wall, circulating platelets & plasma-coagulation proteins

1. Constriction of blood vessels to limit blood loss
2. Contribution from platelets & coagulation proteins → thrombus (blood clot)
3. Thrombi & emboli formation – dangerous → occlude blood vessels, obstruct flow of blood through circulatory system & deprive tissue of O₂ and nutrients
   a. Thrombus: clot adheres to interior wall of an artery or vein
   b. Embolus: blood clot that has been dislodged & travelling through blood stream

Arterial thrombosis: PRIMARY HEMOSTASIS – occurs w/in seconds; important in stopping blood loss

1. Injury → vasoconstrictions (endothelin-1) → local spasm of smooth muscle wall → local vasoconstriction
2. Primary hemostasis: platelets provide initial hemostatic plug at site of vascular injury
   ▪ NORMAL: platelets don’t adhere to healthy arterial walls nor activated by vascular endothelium
     → Platelets & endothelial cells have negatively charged glycoprotein charge = REPULSION
   ▪ DAMAGE to blood vessels: exposes collagen & other underlying tissue with POSITIVE CHARGE
3. Platelet adhesion: loss of intact endothelial lining of blood vessels & exposure of platelet to activating sub-endothelial structures (collagen) → platelet adhering to collagen fibrils in vascular sub-endothelium
   • Adhere to collagen via specific platelet collagen receptor made up of glycoproteins (Gp)
     o GPVI: central receptor – facilitates direct contact with sub-endothelial collagen (also
       ▪ Also: α2β1-integrin & GPIa
     o GPIb: indirectly interacts with collagen via binding to von Willebrand factor (vWF)
       ▪ vWF: unique adhesive glycoprotein
         ▪ Released from injured endothelial cell & platelets
         ▪ Allows platelets to remain attached/anchored to vessel wall despite high shear forces generated within vascular lumen
4. Platelet activation: initial adherent platelets undergo activation (degranulation)
   a) Binding of platelets to collagen (MOST IMPORTANT) or vWF → calcium mobilization from intracellular stores (DTS – dense tubular system)
   b) Calcium promotes platelet shape change → movement of granules
   c) Prepackaged platelet granules contain second-wave agonists (TXA₂ & ADP) released = degranulation
      → Further enhance platelet activation by binding to respective platelet receptors (TXA₂ → TP receptor; ADP → P2Y₁₂ receptor) to increase intracellular calcium even more, or to activate ADP & TXA₂ on secondary platelets
   d) Robust increase in calcium stimulates cyclooxygenase to promote synthesis of TXA₂
      → Calcium activates phospholipase A₂ → cleaves phospholipids & liberates arachidonic acid → arachidonic acid + COX = prostaglandin H₂ → metabolism of PGH₂ facilitated by thromboxane synthase = thromboxane A₂
2. Platelet aggregation: second wave chemical mediators expose platelet surface receptors (conformational change when increase in calcium) to promote platelet aggregation * ytombus growth
   ▪ Predominant receptor is GPIIbb/IIIa (aka integran αIIbβ3) → facilitates contact with circulating proteins (mainly fibrinogen, also vWF)
     → Fibrinogen acts as bridge between two platelets (RGD recognition sequence on each end of fibrinogen; RGD = arginine-glycine-aspartic acid)
     → Platelet-fibrinogen linkages rapidly enlarge platelet plug → primary hemostatic plug
3. Platelet plug is not stable & can be dislodged → secondary hemostasis begins (several minutes)
**Arterial thrombosis: SECONDARY HEMOSTASIS** – plasma coagulation → fibrin clot – strengthens 1° clot

### Components of the coagulation cascade
- Protease (from preceding stage)
  - HMWK (high MW kallikrein) & prekallikrein
  - Factors XII, XI, IX, X, VII & II (prothrombin)
- Precursor protein (synthesized in liver; normally inactive)
- Non-enzymatic protein cofactor (reaction accelerator)
  - Factors V, VIII & tissue factor (thromboplastin)
- Ca$^{2+}$
- Organizing surface (phospholipid surface; in vivo activated platelets)

### Final common pathway
1. Conversion of prothrombin → thrombin (by factor Xa)
2. Fibrinogen (soluble) → fibrin (insoluble) – in presence of thrombin (aka factor IIa)
3. Fibrin covalent cross-bridging = white string-like substance, traps RBCs in a fresh clot
   a. RBCs held together by fibrin

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### Two major pathways: intrinsic & extrinsic systems – converge at final common pathway

#### Intrinsic system
- All clotting factors present within blood vessels
- Activation of factor XII on contact w/ negatively charged surface or exposed collagen fibres initiates the intrinsic coagulation system
- Slower – several minutes

#### Extrinsic pathway
- Initial stimulus = tissue factor – present outside blood vessels
  - Damage to blood vessels exposes TF containing cells from underlying layers to blood stream
  - TF (membrane-bound cell surface glycoprotein) binds in presence of Ca$^{2+}$ to factor VII (circulating in blood stream)
    - Sets off sequential protease activations
    - Faster (within seconds)
- PATHOLOGICAL CONDITIONS: TF also expressed on circulating leukocytes, possibly activated endothelial cells, microvesicles (fragments of membrane from activated platelets & leukocytes) → formation of venous clots

### Additional notes
- Thrombin (IIa) can also strongly activate platelets → granule release & platelet aggregation
- Thrombin can also bind to thrombomodulin expressed on EC surfaces → cleaves & activates protein C (in presence of co-factor Va)
- Protein C cleaves & inactivates Va & Vill (with cofactor Protein S) inhibits clotting = natural anticoagulation
- Antithrombin = endogenous anticoagulant
**Venous thrombosis**

1. Valves in veins → turbulent flow → reduced oxygenation of valve endothelium → valve pocket sinus becomes hypoxic
2. Hypoxic valve pocket sinus → surface expression of adhesion proteins (P-selectin, E-selectin, vWF)
3. Circulating leukocytes, platelets, TF+ microvesicles, bind to activated endothelium (via P-selectin glycoprotein ligand-1 aka PSGL-1) → activated leukocytes that express TF
4. TF binds to factor VII → VIIa and forms TF-FVIIa complex → activation of factor X → Xa
   a) Initiates coagulation process → thrombin
   b) Local activation of coagulation cascade overwhelms protective anticoagulant pathways & triggers thrombosis
5. Once formed, thrombus may extend along vessel & embolize

Valve pocket thrombi contain mostly red cells & fibrin → management centered on anticoagulation strategies

### Factors contributing to formation of thrombus in deep vein thrombus formation
- Reduced blood flow & stasis (surgery, hospitalization, paralysis, long-haul travel, pregnancy)
- Thrombophilia or procoagulant changes in blood
  - Body makes too much blood-clotting protein (Prothrombin)
  - Blood clotting protein (Factor V) doesn’t function well – can’t be inactivated by Protein C & S
- Activation of endothelium: low expression of anticoagulant protein thrombodulin & high expression of procoagulant protein TF

### Anticoagulant: VITAMIN K ANTAGONIST – Warfarin or Nicoumalone = coumarin derivatives

**MOA:** anticoagulant effects only present in vivo
- Interferes with hepatic synthesis of vit K-dependent clotting factors: factors II, VII, IX, X
  - Also interferes with proteins C & S (early effect) → increases coagulation and is overcome by simultaneous heparin administration
- Vit K (reduced) → Vit K epoxide (oxidized) during synthesis of these clotting factors
- Vit K epoxide reductase reduces the epoxide back
  - Vit K antagonists inhibit this reductase → decreasing active form of Vit K (antagonizes Vit K recycling)

### Drug info
- Peak concentration: 2-8 h; duration of action: 4-7 days
- T½: 25-60 h
- Highly plasma protein bound → small Vd
- Metabolized by liver – *genetic variances & drug interactions*
  - Substrate of CYP2C9 (major) & CYP2C8 (minor)
- Effectiveness decreased if taken with Vit K rich foods (green veggies, beef liver, green tea)
- Warfarin doesn’t affect previously synthesized factors → anticoagulant effects not observed until 8-12 h after administration
- Effective anticoagulation requires ~ 1 week
- ADRs: hemorrhage (blood in stools/urine, excessive nose bleeds/bleeding gums)
  - Minor bleeding treated by withdrawal of drug & vit K (oral)
  - Severe bleeding: high dose of Vit K (IV) or transfusion of fresh plasma
- NOT used in pregnancy: birth malformation & abortion (cross placenta)

### Monitoring
- Prothrombin time: 12-14 seconds
  - Clotting after recalcification shortened to 12-14 sec by addition of phospholipids & thromboplastin
- INR = PT ratio (test/control) allowing for comparison b/w labs
  - Goal INR 2-3
Pharmacology of Anticoagulants

**Anticoagulant: INDIRECT THROMBIN INHIBITORS** - prevent blood coagulation by binding to antithrombin

### Heparin
- Occurs in secretory granules of mast cells; extracted from pig intestinal mucosa or bovine lung (DC)
- Strongly anionic – sulfate & carboxyl groups

#### Unfractioned heparin (UFH)
- Injectable (deep SC or IV injection/infusion)
  - Not IM – causes hematomas
- Rapidly acting anticoagulant (peak after 2-4 h)
- Used when immediate anticoagulation required
  - Anticoagulant of choice for pregnant women
  - Also prophylaxis to prevent DVT or PE (prior to hip-joint replacement, renal dialysis, coronary & vascular surgery, cardiopulmonary bypass)
- No intrinsic anticoagulation activity → binds to plasma antithrombin III
  - ATIII = plasma protease inhibitor synthesized in liver & circulates plasma → slowly inhibits thrombin & factor Xa
    - In absence of heparin: rate of thrombin inactivation by antithrombin relatively low
  - Binding of heparin → conformational change in ATIII that accelerates interaction w/ thrombin & Xa by 1000-4000 x
    - After this, heparin dissociates from ATIII → activate further ATIII molecules
- Complex pentasaccharide-ATIII sufficient to inhibit activation of factor X
- To inhibit thrombin, pentasaccharide must be included onto a chain of 18 monosaccharide units or more (MW > 5400)
- Also bind plasma proteins, endothelial cells & macrophages

#### Monitoring
- Continuous IV infusion: activated partial thromboplastin time (aPTT)
  - Blood clots 4-8 min in vitro
  - Recalified plasma clots in 2-4 min
  - aPTT determines clotting time after recalcification & addition of phospholipids (negatively charged PO₄ groups) – shortened to 26-33 seconds
  - Therapeutically, clotting time of 1.5 – 2.5 times aPTT value is aimed for (45-70 seconds)
  - q4-6 h after commencing infusion
- SC: only initial monitoring necessary

#### Low molecular weight heparins (LMWH)
- Heparin depolymerized into distinct fragments on basis of MW (4000-6000; approx. 17 saccharide units)
- Better bioavailability (less binding to endothelium & plasma proteins)
- Longer-half-life (slow hepatic clearance)
- Predictable anticoagulant response (fixed doses without monitoring = outpatients)
- Threefold higher ratio of anti-Xa to anti-thrombin activity than UFH
- Administration by SC injection
- Excreted in urine → renal insufficient patients
  - UFH given

#### Adverse effects
- Bleeding: may be managed by slow IV infusion of protamine sulfate (1 mg binds to 100 units of heparin)
  - Less effective against LMWH
- Allergic rxns (chills, fever, itching)
- Heparin-induced thrombocytopenia (HIT): predisposes to formation of abnormal blood clot (paradox)
  - Antibody formation against platelet factor 4-heparin complex → low platelet count (destruction) & act as antagonists to activate platelets → release MVs → activate thrombin → thrombosis
  - More common in high dose UFH + osteoporosis w/ chronic therapy
New oral anticoagulant (NOAC): DIRECT THROMBIN INHIBITORS – bind directly to thrombin’s catalytic site \(\rightarrow\) block conversion of fibrinogen to fibrin; inhibit both circulating & clot-bound thrombin (which usually activate & recruit more platelets)

Dabigatran: specific factor IIa (thrombin) reversible inhibitor
- Selective – lack of effect on other serine proteases involved in coagulation
- Prodrug \(\rightarrow\) converted to active dabigatran by non-specific esterases in blood (and liver)
  - Low bioavailability (6.5%)
  - Significant re-secretion after absorption in gut (as for all NOACs)
- Don’t bind platelet factor 4 \(\rightarrow\) no risk of HIT
- Not metabolized by CYP450 system \(\rightarrow\) least likely to have drug interactions
  - Elimination mainly renal (80%)
- Fixed oral dose (twice daily) w/o need for INR (coagulation monitoring)
  - Max anticoagulant activity 2-3 h after ingestion
  - \(T_{1/2}\): 12-17 h (relatively short)

NOAC: FACTOR Xa INHIBITORS: target factor Xa, preventing conversion of prothrombin to thrombin

Rivaroxaban (DIRECT): competitively & selectively directly inhibitors factor Xa
- Binds to catalytic/active site of factor Xa & directly interferes with coagulation cascade
  - Results in prolongation of clotting times in human plasma
- Inhibition for factor Xa is 10,000 x greater than for other serine proteases
- Predictable PK \(\rightarrow\) fixed oral dosing w/o need for INR monitoring
  - Once daily dosing (mandatory with evening meal)
- Max time to onset rapid (2.5 – 4 h)
- \(T_{1/2}\): under 12 hr
- Elimination direct renal (1/3) & fecal-biliary route after metabolism via hepatic CYP3A4

Apixaban (DIRECT): potent, reversible, direct inhibitor of factor Xa
- 30,000x selectivity over other proteases
- Max time to onset rapid (3h)
- \(T_{1/2}\): 8-15 h
- Elimination: 25% renal, 75% fecal
  - Minor CYP3A4 hepatic clearance
- Twice daily dosing w/ no INR
  - Food doesn’t affect absorption
- Drug interaction: strong

Edoxaban: FDA approved, waiting for Health Canada approval

Fondaparinux (INDIRECT): binds to antithrombin & enhances ability to inhibit factor Xa by 300x
- Parenteral SC administration (once daily dosing – long \(t_{1/2}\))
- Synthetic, w/ heparin pentasaccharide sequence necessary for binding to ATIII
- ADR: bleeding (doesn’t have thrombocytopenia like heparin)
- Excreted unchanged in urine

<table>
<thead>
<tr>
<th>NOAC practical advantages</th>
<th>NOAC limitations</th>
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<td>Few known or defined drug interactions</td>
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