Hemostasis

- what is hemostasis?
  hemostasis is the blood clotting process that prevents excessive hemorrhage after blood vessel damage.
- describe the sequence of events leading to hemostasis at the site of vascular injury
  1. **Arteriolar vasoconstriction**: is immediate and transient. Mediated by neurogenic reflex, and augmented by *endothelin *****(EC-secreted potent vasoconstrictor) and other similar factors.
  2. **Primary hemostasis: formation of primary platelet plug.** The damaged endothelium exposes subendothelial **collagen** and **Van Willebrand factor (vWF).** Platelets bind via **GpIb** receptors to vWF on exposed ECM and are activated (shape change + granule content release). Platelets release secretory granules containing (among other things) **ADP**, and **Thromboxane A2 (TxA2)** that recruit additional platelets and induce platelet aggregation through platelet **GpIIb-IIIa** receptor binding to fibrinogen.
  3. **Secondary hemostasis**: The exposed subendothelial cells (fibroblasts, SMCs) express a membrane-bound procoagulant glycoprotein, **Tissue Factor**, which activates **Factor VII**. Factor VII activation generates a coagulation cascade that culminates in the formation of **Thrombin**. Thrombin has two roles: it is a potent platelet activator, so it promotes additional platelet aggregation at the site of injury, and it also cleaves fibrinogen into fibrin. **Fibrin** creates a meshwork that stabilizes the clot.
  4. **Clot stabilization and resorption**: the clot (platelets and polymerized fibrin) contracts to become a solid, permanent clot. At this point there is release from the regenerated endothelium of **thrombomodulin** that blocks the coagulation cascade, and **tissue plasminogen activator (t-PA)** that promote fibrinolysis and clot dissolution.
- in general, what are the features of platelets that confer their functions?
  Platelets have
  - several **glycoprotein receptors**
  - **a contractile cytoskeleton**
  - **alpha granules** containing
    - adhesion molecule **P-selectin** on the membranes of the granules
    - coagulation proteins including **fibrinogen, coagulation factor V, and vWF.**
    - protein factors involved in wound healing: **fibronectin, platelet**

factor 4, PDGF, TGF-beta.
    - **dense (delta) granules** containing
        - ADP
        - ATP
        - Ca++
        - serotonin
        - epinephrine
- what intrinsic properties of platelets allow platelet adhesion?
    - interaction between membrane-bound **Glycoprotein Ib** (GpIb) in platelets with **Von Willebrand factor** in the subendothelium (that is in turn bound to collagen, so it acts as a bridge).
      Deficiency in Von Willebrand causes Von Willebrand disease.
      Deficiency in GpIb causes Bernard-Soulier syndrome. Both are bleeding disorders.
    - direct binding to type I and IV **collagen** via **Glycoprotein Ia / IIa complex** (GpIa/IIa)
- what allows platelet aggregation?
    - **Aggregation is accomplished by GpIIb-IIIa receptors on different platelets binding fibrinogen**
    - This receptor is “activated” (it acquires high affinity for fibrinogen) by the conformational change that takes place when the platelets are activated.
- ❓
- what triggers platelet activation?
    - Platelet activation is trigger by (among other things) **Thrombin and ADP**
        - **Thrombin** activates platelets by activating GPCR called **protease-activated receptor-1 (PAR-1)** by proteolytic cleavage.
            - Initial wave of aggregation is reversible (with the activation of thrombin, the platelet plug will be irreversible)
        - **ADP** binds **P2Y(1) and P2Y(12)** receptors (GPCRs) → **platelet recruitment**
    - activated platelets produce **Thromboxin A2 (TXA2)** (generated from prostaglandin H2) which is in turn a potent inducer of **platelet aggregation**.
- what are the changes in platelets upon their activation?
    - Upon activation platelets:
        - release of alpha and delta granules: Ca++, ADP (further platelet activation), coagulation proteins, wound healing factors, etc.
        - change their shape (thanks to their contractile cytoskeleton), with that there’s
            - conformational change of the membrane bound glycoprotein IIa/IIIb that increases its **affinity for**

fibrinogen
        - translocation of phosphatidylserine to the outer membrane surface that binds Ca++ and this serves as a **nucleation site** for the assembly of coagulation factor complexes
- describe the coagulation cascade
  it's a series of Ca++ dependent reactions that activate coagulation factors, and culminates in the formation of fibrin and consequently the deposition of insoluble fibrin clot.
  1. Platelet activation → negatively charged phospholipids to the platelet surface and release of Factor V from alpha granules.
  2. **Factor V** binds to phospholipids and acts as a **cofactor for factor Xa. Factor Xa converts Prothrombin → Thrombin. Thrombin converts Fibrinogen → Fibrin clot.**
  3. The formation of factor Xa is the convergence point between two possible pathways:
     1. **Intrinsic pathway:** (AP→ 12→11→9+(8)→ 10) activated (by platelets) factor XII activates factor XI which activates factor IX, which in turn, with the help of activated cofactor VIII, activate factor X.
     2. **Extrinsic pathway:**
        In vitro: (TF+ 7 → 10) Tissue factor acts as a cofactor in the reaction where proteolytic enzyme (factor VIIa) activates factor X.
        In vivo: (7+ TF → **9+8** → 10) tissue factor and factor 7 first activate factor IX, which then activates factor X with factor VIII as a cofactor.

     3. ❓
- describe the functions of Thrombin
  - stimulates **platelet recruitment and degranulation** (aggregation and activation)
  - participates in the generation of **cross-linked fibrin**:
    - fibrinogen (soluble) → fibrin monomers
    - activates factor XIII that covalently cross-links fibrin
  - amplifies coagulation cascade by **activating** several **factors (XI, V, VIII, XIII)**
  - it activates protease-activated receptors (**PARs**) expressed on many cells, contributing to ****
    - in platelets: TxA2 production → platelet aggregation
    - in ECs: expression of adhesion molecules and a variety of fibrinolytic (tPA), vasoactive (NO, PGI2), and cytokine mediators (PDGF)
      - **tissue repair and angiogenesis**
    - leukocytes: activation

    - **Anticoagulant effects**: it can acts as anticoagulant preventing clotting from extending beyond the site of the vascular injury
- describe the fibrinolytic cascade
  Activation of the coagulation cascade also sets into motion a **fibrinolytic cascade** (24h later) ****that activates **Plasmin and as a result it limits the size of the clot and contributes to its later dissolution.**
  Plasmin breaks down fibrin, fibrinogen and some coagulation factors, interferes with fibrin polymerization.
  Plasminogen (found inside the clot) is hydrolyzed into **PLASMIN** by
    - Mainly **t-PA** (tissue plasminogen activator) secreted by ECs
    - urochinase, secreted by ECs
    - kallikrein (plasma enzymes) or factor XII-dependent pathway (possibly explaining the link between factor XII deficiency and thrombosis)
    - or by microorganism-derived proteins (streptokinase, staphylokinase)
- After activation, plasmin is controlled by:
    - **Plasminogen activator inhibitors (PAI):** main inhibitor of tissue-type plasminogen activator (tPA) and urokinase (uPA),
    - **α2-plasmin inhibitor** (plasma protein: binds and inhibits free plasmin)
    - **α2-macroglobulin** (inhibits plasmin, kallikrein and thrombin)
    - and the immune response.
- what ensures the coagulation is confined to the site of injury?
  Release of t-PA (fibrinolysis) and thrombomodulin (blocks coagulation cascade) confine the hemostatic process to the site of injury.
- how does the endothelium prevent thrombosis and limit clotting to sites of vascular damage?
  Normal conditions, the ECs expresses factors that
    - **inhibit platelet aggregation and activation**:
        - Prostacyclin (also called prostaglandin I2 or PGI2) It inhibits platelet activation and is also an effective vasodilator.
        - NO
        - ADP
    - **inhibit coagulation**
        - thrombomodulin
        - endothelial protein C receptor
        - heparin-like molecules (TF pathway inhibition)
        - t-PA (fibrinolysis)
    - ECs also bing thrombin and alter its activity
- what sort of disorders arise from deranged hemostasis?
    - hemorrhagic disorders
    - thrombotic disorders

- describe defects of primary hemostasis
  Defects of primary hemostasis are caused by platelet defects, vessel defects or von Willebrand disease.They can be genetic or acquired.
  - **impaired vasoconstriction**
    - degenerative → amyloidosis, elastosis
    - vessel malformation
  - **von Willebrand disease:** most common blood clotting disorder
  - defects in platelet function
    - **Plateletosis**: N°of platelets above 600,000/µl (myeloproliferative syndromes. Platelets have functional and morphological alterations.
  - **thrombocytopenia** low number of platelets below 100,000 (nv 150,000- 400,000/µL)
- describe defects of secondary hemostasis
  - **hemophilias** (genetic deficiency of coagulation factors)
  - defects in coagulation factor production
    - hepatic diseases
    - anticoagulant therapies (vit.K antagonists, heparin, etc)
  - excessive destruction (e.g. disseminated intravascular proteolysis)
  - defects in receptors (thrombin receptors)
  - others
- describe diseases of the fibrinolytic system
  Components of the fibrinolytic system: plasminogen, plasminogen activators, inhibitors of plasminogen activators, specific plasmin inhibitors (alpha2-antiplasmin, alpha2- macroglobulin)
  - **Genetic or acquired deficiency of plasmin inhibitors** (hyperfibrinolytic syndromes) → excessive fibrinolysis → hemorrhage
  - **Anti-protease deficiency (prevalence of venous thrombosis, thromboembolism**): Main antiproteases (control of proteases and factors VIII, V, XII):
    - alpha1-antitrypsin, alpha1-antichymotrypsin, antithrombin III, protein C and S, C1 inactivator, protein C inhibitor
  - **Genetic or acquired** (liver diseases, carcinomas, leukemia, sickle cell disease, hyperfibrinolytic syndromes, oral contraceptives) **deficiency of antithrombin III: recurrent venous thrombosis**