# THROMBOCYTE STRUCTURE AND THROMBOCYTE-ENDOTHELIUM INTERACTION

Hematopoietic System and Disorders (MED202)

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# Lecture outline

- General characteristics of thrombocytes
- Ultrastructural features of thrombocytes
  - Plasma membrane receptors, adhesion molecules, dense tubular system, etc.
  - Granules of thrombocytes
- Molecular ligands that mediate Thrombocyte-Endothelium interaction
- Adhesion, activation, granule secretion  and aggregation

# Thrombocytes

- More commonly known as **platelets**
- Circulating cell fragments that derived from stem cells found in the BM
- Non-nucleated, cannot replicate
- Normal count: 150,000-350,000 /µl
- Have a really short lifespan: 8-9 days

# Maturation of megakaryocytes

- Several rounds of chromosomal duplication without cell division (up to 64-fold): **endomitosis**
- Results in a single polylobulated nucleus
- Cytoplasmic volume increases proportionately with ploidy
  - used for protein and lipid synthesis in order to support platelet production

# Maturation of megakaryocytes

- İs filled with platelet-specific
  - ✓Organelles
  - ✓Granules
  - ✓Cytoskeletal proteins
  - ✓Invaginated membrane system
    - constitute an extensive internal membrane reservoir that may facilitate platelet production

# After maturation

- Megakaryocytes migrate to the vascular niche
- Extend, through junctions in the lining of blood sinuses, long branching processes **proplatelets**.
  - Tubulin and actin

- Slender tubular projections with platelet-sized swellings at their
  ends

# Platelet morphology and ultrastructure

- Not true cells but merely circulating fragments of cells.
- Small: 2-3 μm
- Anucleate
- Disc-shaped
- 2/3 in circulation
- 1/3 reversibly sequestered in the spleen

# Four distinct zones

1. Peripheral zone
2. Structural zone
3. Membrane systems
4. Organelle zone

# Peripheral zone

- ✓Covered by a thick surface coat of Glycocalyx
  - ✓Adhesion and aggregation
  - ✓Cell's negative charge
- ✓Contains receptors
  - ✓**GPIb-IX-V**: major platelet receptor for vWF
  - ✓**GPIa/-Ia** and **GPVI**: recetors for collagen
  - ✓**GPIIb-IIIa**: receptor for vWF
  - ✓**Thromboxane A2 (TXA2) receptor**
  - ✓**P2Y12** and **P2Y1**: Receptors for ADP
- ✓Membrane lipids
  - ✓Resposible for the release of fatty acid derivatives: PGs, prostacyclins

# Structural zone

- ✓Organized network of protein filaments
- ✓Maintaining discoid shape while resting
  - a spectrin-based membrane skeleton
  - an actin-based cytoskeletal network
  - a peripheral band of microtubules

# Membrane Systems

- Structure and support
- **Open canalicular system (OCS)**
  - ✓Secretion of granule cotents
  - ✓Provides a route for entry and secretion
  - ✓Filopodia formation and spreading following platelet adhesion to an activating surface
- **Dense tubular system (DTS)**
  - ✓Storage of $Ca^{2+}$
  - ✓Prostaglandin and thromboxane synthesis

# Organelle zone

- ✓Secretion and storage
- ✓Mitochondria
- ✓Lysosomes
- ✓Peroxisomes
- ✓Glycogen particles
- ✓**Granules:** serve as storage sites for proteins and other substances necessary for platelet function

  - Dense granules
  - α-granules

# Granules: Dense granules

- 4-8 per platelet
- Contain **platelet agonists** and signaling molecules that amplify platelet activation
  - Non-protein substances that are secreted in response to platelet activation
    - Serotonin
    - ADP, and other adenine nucleotides
- High concentrations of calcium and phosphate

# Granules: α-granules

- 50–60 per platelet
- contain proteins that enhance adhesion, growth factors, and coagulation factors
  - **Adhesive proteins**
    - Fibronectin
    - vWF
    - Thrombospondin
    - Vitronection
    - P-selectin
  - **Growth factors**
    - PDGF
    - TGF-β
    - Platelet factor 4
    - VEGF
  - **Coagulation factors**
    - Factor V
    - C1 inhibitör
    - Factor XI
    - Factor XIII
    - Protein S
    - PAI-1

# Platelets

- Circulate within the blood itself
- serve as the “band-aids” of the bloodstream
  - respond to blood vessel injury
    - changing shape,
    - secreting their granule contents
    - aggregating to form a platelet clot.
- Platelets also play secondary roles
  - Helping regulate angiogenesis
  - Vasomotor function
  - Chemotaxis
  - Inflammation
  - Atherosclerosis
  - Innate immunity

# Endothelium

- The entire vascular system is covered by a single strut of endothelial cells
- In the past: simple passive barrier
- Now: as an organ whose normal functioning is crucial for maintaining vascular health

# Endothelium

- Semipermeable
- Regulates transport of macromolecules between the vascular lumen and vascular smooth muscle.
- Maintain an undisturbed blood flow under physiologic conditions.
- Regulates
  - vascular tone
  - thrombosis and thrombolysis
  - platelets adherence and activation

# Under physiologic conditions platelets circulate without adhering to intact and inactive endothelium.

- A layer of proteoglycans and glycoproteins is present between ECs and blood: **Glycocalyx**
- Regulates endothelium permeability and endothelium interactions with other cells (eg. platelets and neutrophils)
  - Repelling them via its negative charge
  - Limiting the endothelial exposure to adhesion molecules

# Unactivated endothelial cells

- Due to their antithrombotic properties, they prevent platelets from sticking to the vessel wall (adhesion)
- They secrete a number of factors that inhibit platelet activation

✓Nitric oxide

✓Prostacycline

✓Cyclooxygenase-2

✓Ecto-ADPase

# Damaged endothelial cells

- Activated
- Gain **prothrombotic properties**
- ECM components (eg. collagen) are exposed
- Secrete molecules that will promote platelet adhesion and activation
- **ADP**, **vWF**, **adhesion molecules**
- vWF specifically mediates the interaction between endothelium and platelets (even under high shear stress)
- **Adhesion** of platelets to ECM is the primary event in thrombus formation

# Platelet-Endothelium Interaction: Molecular Ligands

The interaction between the two cells is mediated mainly by **lectins** and **glycans**

# Lectins

- They are classified into 5 different groups according to their structural features.
  - **C-type lectins**
    - **P-selectin**
  - **I-type lectins**
  - **S-type lectins (galectins)**
  - Pentraxins
  - P-type lectins

# P-selectin

- A key element that plays an important role in the platelet-endothelium interaction
- Platelets: α-granules
- Endothelium: Weibel-Palade bodies
- Immediately after the cell is activated, they are localized to the cell membrane
- Acts as a cell adhesion molecule
- Mediates leukocyte adhesion to the endothelium and rosette formation of platelets on leukocyte-endothelial cells.

# Receptors for P-selectin

- Platelet glycoprotein Ib alpha chain (**GP1b-alpha**): on platelets
- P-selectin glycoprotein ligand-1 (**PSGL-1**): on both platelets and leukocytes
- Sulphatide
- Glycosilation-dependent cell adhesion molecule (GLYCAM)
- Endoglycan

Platelets can bind to the intact endothelium;

- partly because the physiological inhibitory mechanisms are impaired
- partly because new adhesion molecules are expressed on the surfaces of activated platelets and endothelial cells

# Platelet Adhesion and Aggregation

- Damage to the endothelial layer leads to the contact of blood cells with the sub-endothelial matrix.
- Various adhesive proteins support the attachment of platelets to the subendothelial matrix.

**Adhesion Activators**

Collagen
Thrombin
ADP: induces aggregation
Thromboxane A2
Calcium
vWF
P-selectin
Vitronectin

# Platelet Adhesion and Aggregation

- Each adhesive protein interacts with the specific platelet surface receptor.
- Adhesion activates platelets and stimulates the secretory response.

# Secretion of granule contents

- Happens by cell membrane invagination towards the platelet center
- Some of the alpha-granules are fused together.
- These combined granules secrets their **contents** via excocytosis.

- **GPIIb-IIIa:** Found on platelet membrane and is a fibrinogen receptor

- **Binding of fibrinogen** to this receptor is the basis of platelet aggregation

| Platelet Function | Agonists, Ligands | Receptors |
|---|---|---|
| Initial and firm adhesion | vWF | GPIb/V/IX |
| | TSP1 | GPIb/V/IX, CD36 |
| | Collagen | $\alpha_2\beta_1$, GPVI, CD36 |
| | Fibrinogen | $\alpha_{IIb}\beta_3$ |
| | Fibronectin | $\alpha_5\beta_1$[73] |
| | Vitronectin | $\alpha_v\beta_3$[77] |
| | Laminin | $\alpha_6\beta_1$[74] |
| | High shear stress | GPIb/V/IX |
| Activation and amplification | Thrombin | PAR1, PAR4, GPIb/V/IX |
| | ADP | $P2Y_1$, $P2Y_{12}$ |
| | $TxA_2$ | TP$\alpha$, TP$\beta$ |
| | Epinephrine | $\alpha_{2A}$ |
| | Serotonin | 5-HT2A |
| | MMP-2, MMP-1[75,76] | ? |
| | Immune complexes | Fc$\gamma$IIa |
| | Complement factors | C1q, C3a, C5a receptors |
| | Plasmin | ? |
| | Streptokinase | ? |
| Aggregation/amplification and stabilization | Fibrin | Activated $\alpha_{IIb}\beta_3$ |
| | vWF | Activated $\alpha_{IIb}\beta_3$, GPIb/V/IX |
| | TSP-1[77] | Activated $\alpha_{IIb}\beta_3$, CD36,IAP |
| | Fibronectin | Activated $\alpha_{IIb}\beta_3$ |
| | sCD40L | Activated $\alpha_{IIb}\beta_3$ |
| | Gas6 | Axl[78,79] |
| | SDF-1, TARC, MDC | CXCR4, CCR4[80–82] |

Platelets: physiology and biochemistry K Jurk, BE Kehrel Seminars in thrombosis and hemostasis. 2005: 31 (04), 381-392

Once they are activated, platelets form clot on the damaged vessel to prevent blood loss.