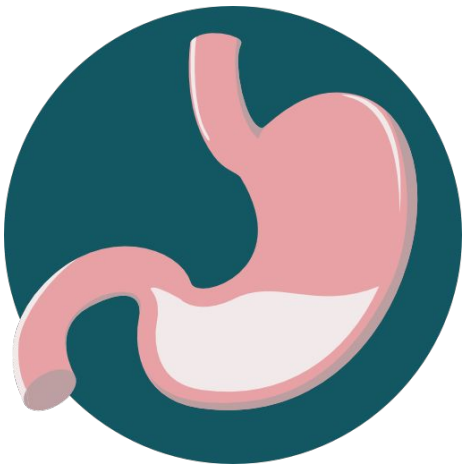
جامعة الملك سعود
King Saud University

MED437
KING SAUD UNIVERSITY



Revised By
Hadeel Awartani Abdullah AlOmar

Platelet structure and function

Objectives:

- Describe formation and development of platelets.
- Understand platelet normal ultrastructure.
- Describe the functions of different platelets organelles and surface receptors.
- Describe the mechanism of platelet functions.
- Relate membrane receptors and granule content to normal function in hemostasis and bleeding (platelet) disorders.

Done by:

- Team leader: Rahaf AlShammari, Abdulrahmen AlHayssoni
- Team members:
  - Yazeed AlKhayyal, Khalid AlMutairi, Abdullah AlSargani
  - Majd AlBarrak, Renad AlMegrin
  - Shahad AlTayash, Hadeel AlMukaynizi
  - AlAnoud AlEssa, Dana AlKadi

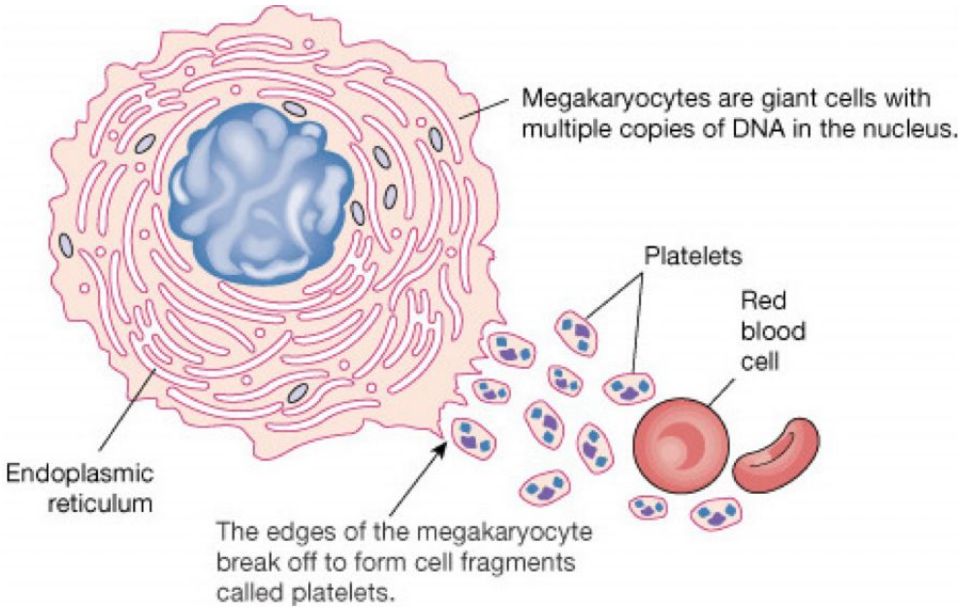
★ Special thanks for Sultan AlNasser!

Colour index:
- Important
- Numbers
- Extra

وَأَن لَّيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَىٰ

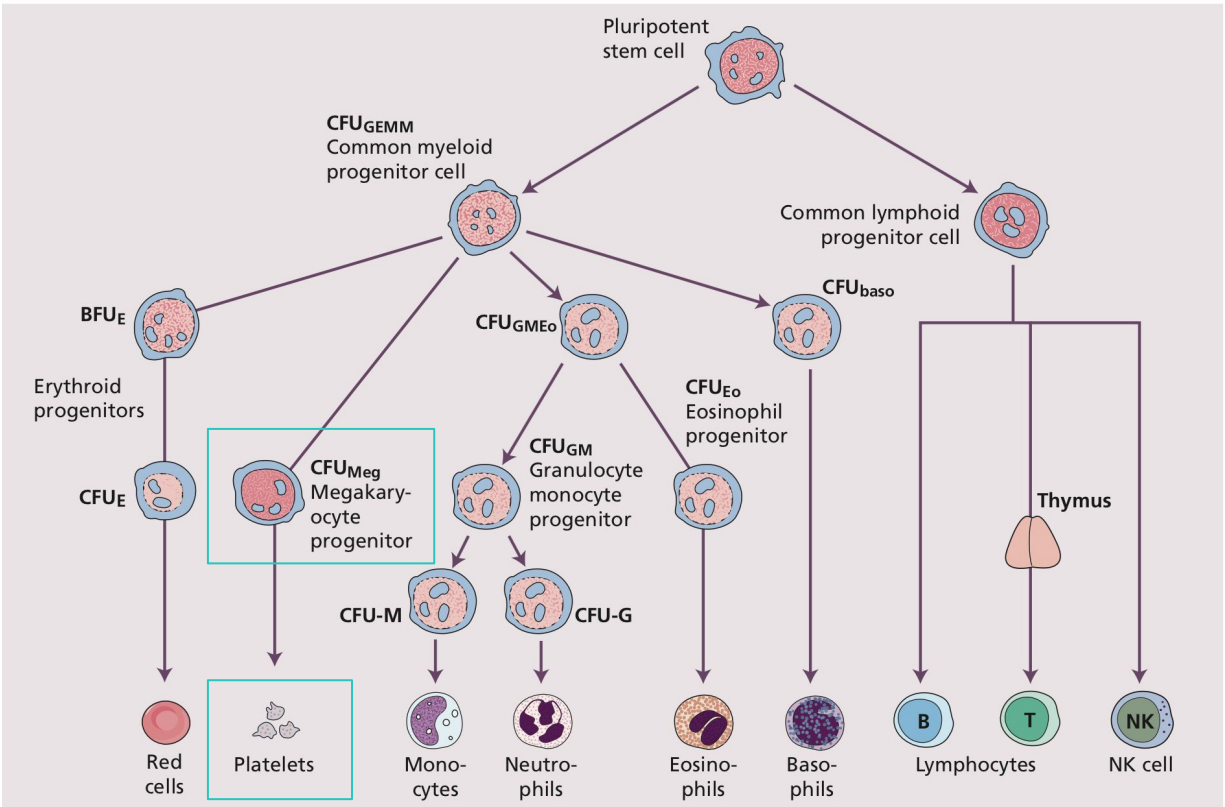
Platelets

Formed by fragmentation from megakaryocytes.

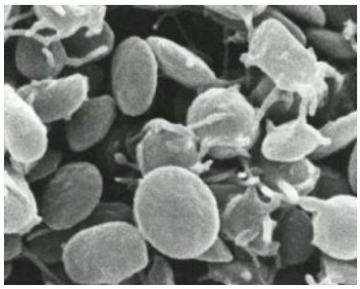


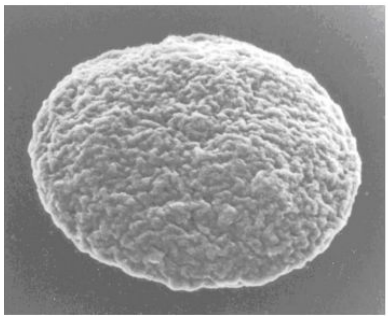
Site of formation

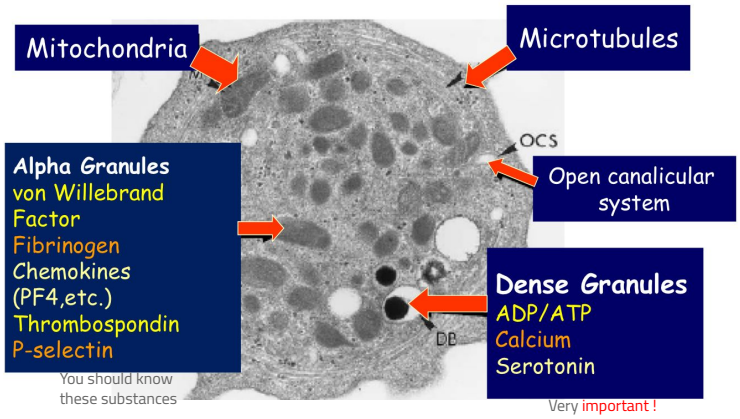
Bone marrow



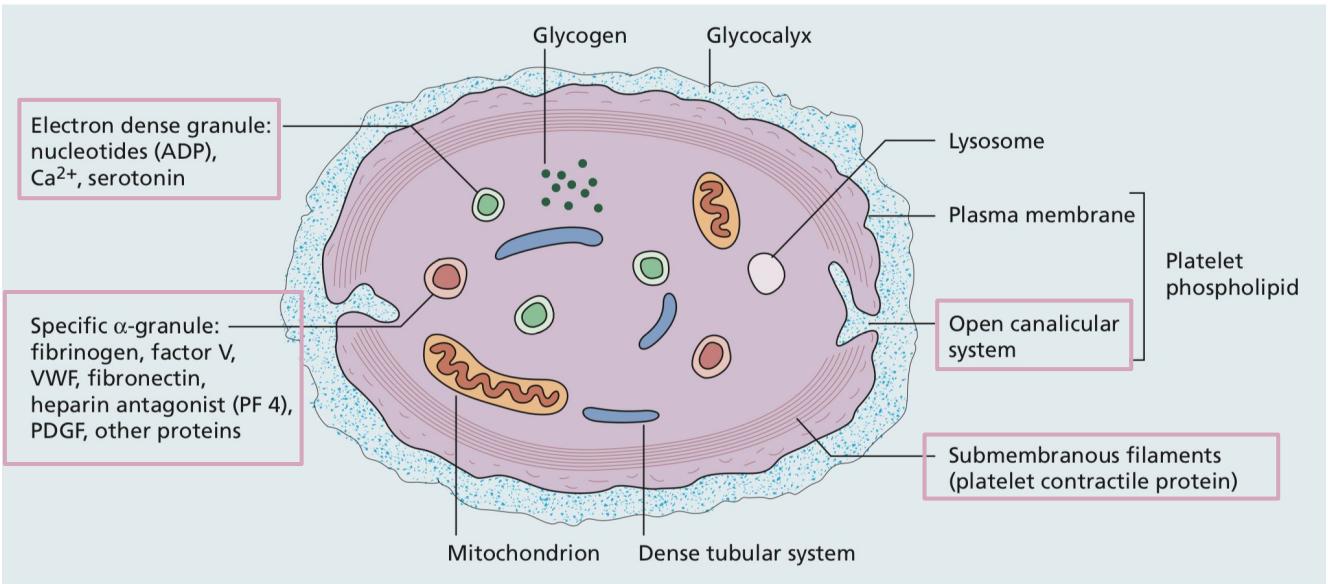
Platelet Ultrastructure







The surface is not smooth in resembles the gyri and sulci of the brain, why? To increase surface area.



Thrombocytes

Nucleus	None (Anuclear)
Shape	Discoid (inactivated) Spherical (activated)
Platelet count	150 x10^3 - 300x10^3/ml
Size	1.5-3.0μm
Life span	7-10 days

- Contractile, adhesive, cell fragments
- Store coagulation factors & enzymes
- Surface binding or antigens glycoproteins
- Sequestered in the spleen; hypersplenism may lead to low platelet count.

⅓ (20%) stored in spleen for emergency,
⅔ (80%) circulating in the blood.

You have to know each part and the contents:

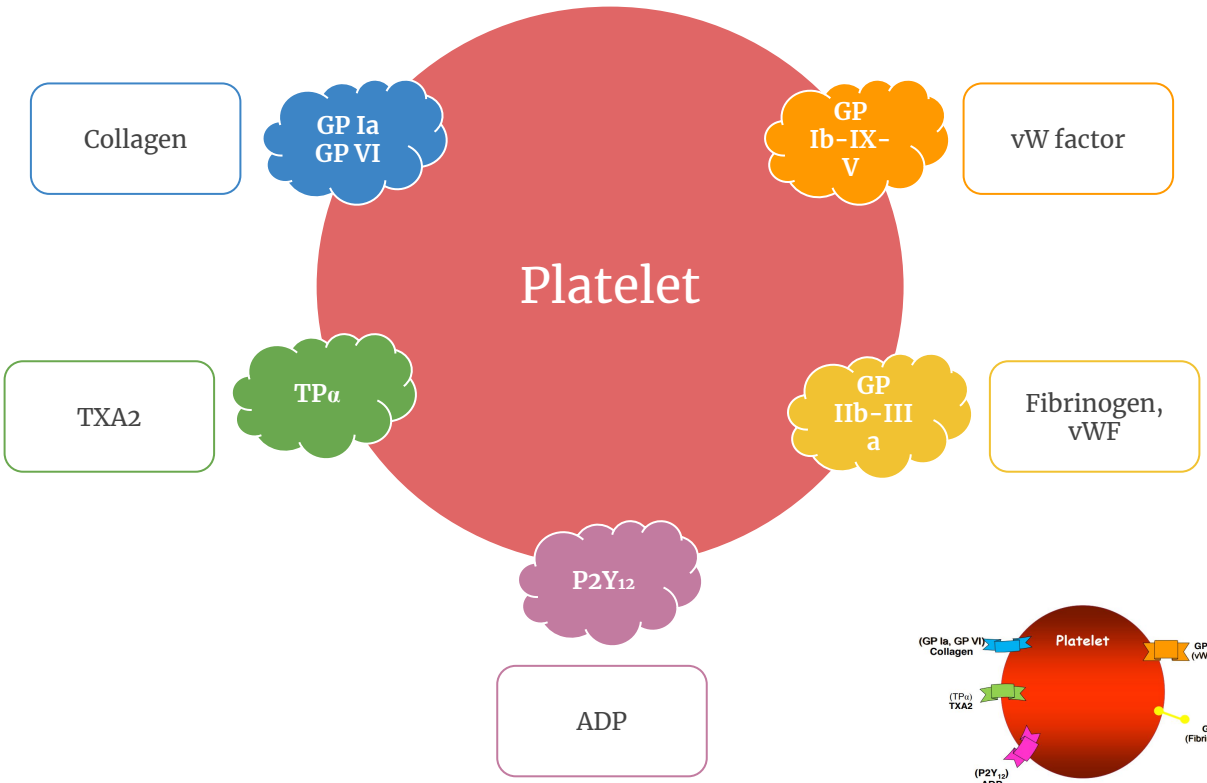
1. Dense (delta) bodies: contain ADP, serotonin, Ca.
2. alpha granules: Coagulation factors: (fibrinogen, vWF), PDGF, PF4, thrombospondin, P-selectin, Chemokines.
3. Open canaliculi system the cell membrane is not continuous,its function to:
   a. Increase surface area.
   b. Secretes the contents of alpha granules.
   c. Stimulus entrance.
4. Microtubules is responsible for the disc shaped and support.

Functional characteristics

Q: What are the functional characteristics of platelets? may come as SAQ!

- **Motile**: Actin and Myosin molecules.
- **Active**: Endoplasmic reticulum, Golgi apparatus and mitochondria.
- **Enzymes** system: synthesis of prostaglandins
- **Granules** (alpha and delta/dense)

Platelet Receptors



1. (GP Ia,GP VI)
   - binds to collagen [it will be exposed under the endothelium in injuries].
2. (GP Ib-IX-V)
   - binds to vW factor (it's a clotting factor from endothelial cells).
3. (GP IIb-IIIa)
   - binds to fibrinogen [its present in α granules & plasma in inactive state, and vWF).
4. (TPα)
   - binds to TXA2.
5. (P2Y12)
   - binds to ADP.

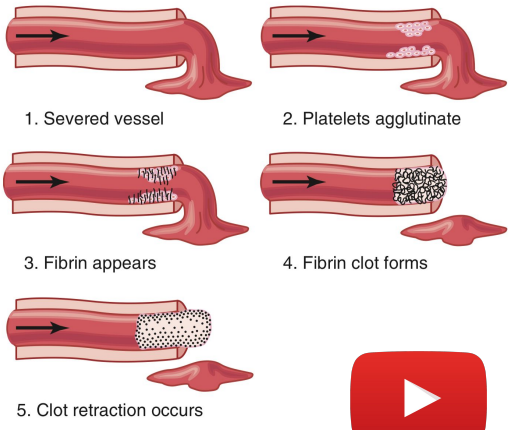
Extremely important!!

You have to memorize the name of each receptor and what does it bind to

General functions of the platelets

Hemostasis

1. Vascular phase
2. Platelet phase
3. Coagulation phase
4. Fibrinolytic phase



Platelet activation

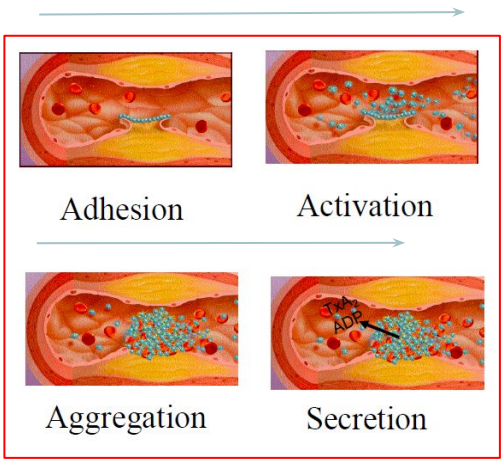
Hemostatic mechanisms

1. Vessel wall
2. Platelet
3. Blood coagulation
4. Fibrinolytic system

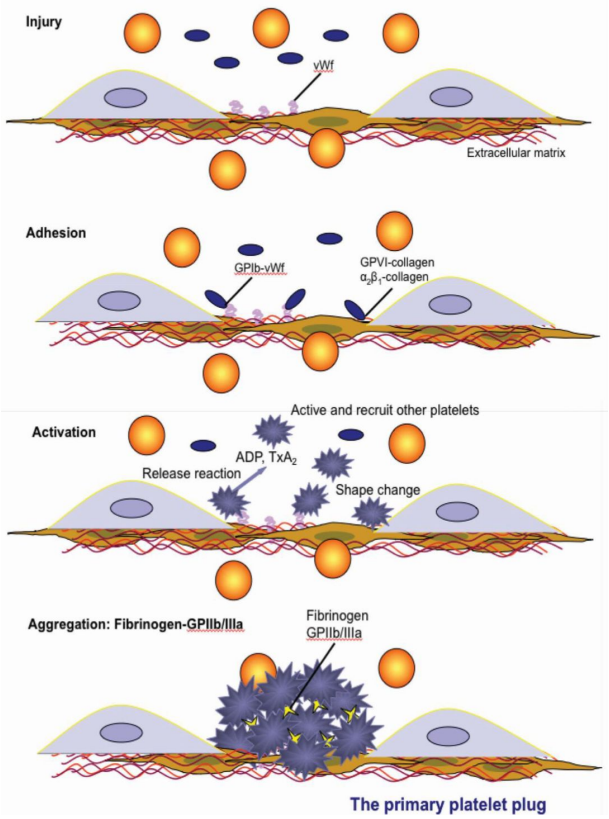
Platelet activation

What's the main function of the platelets? Initial arrest of bleeding by formation of primary hemostatic plug, the mechanism is:

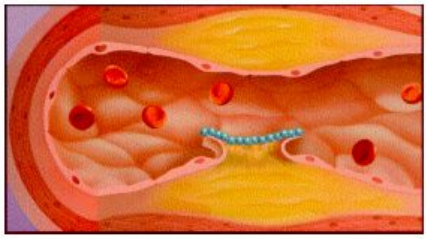
Adhesion → Shape change → Aggregation → Release reaction → Clot retraction



SAQ
The sequence is very important



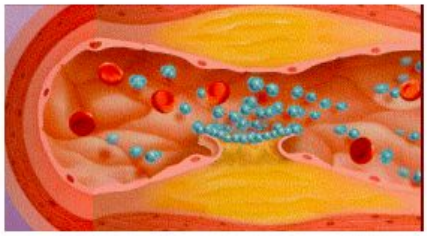
1. Adhesion *1

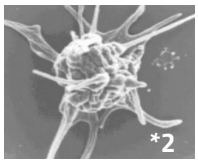


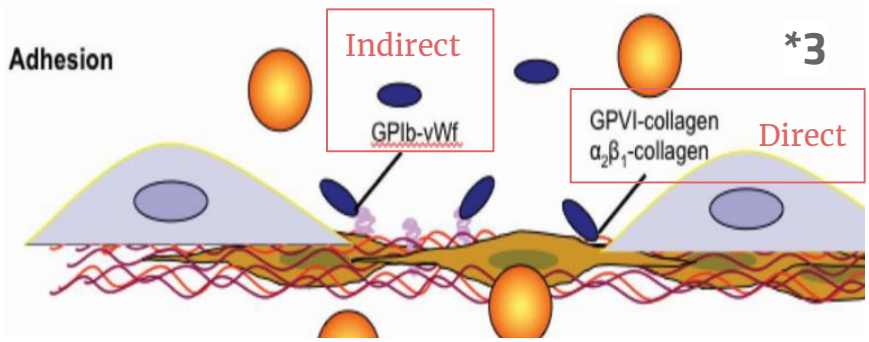
1- Adhesion and 2- shape change [activation] phases:

- Platelets stick to exposed collagen underlying damaged endothelial cells in vessel wall (pic. **1**, the platelets are blue).
- Platelets are activated by adhesion > extend projections to make contact with each other (pic **2**).
- **Adhesion** (interaction between platelet and subendothelial tissue[collagen]), happens by 2 ways (pic **3**):
  a. Directly: when there's injury, the collagen explode (there's strong attraction between platelet and collagen, so as long as the collagen covered the platelet won't adhere endothelial cells, and when there's explosion there will be uncover to the collagen and the attraction will happen (binding by coreceptor).
  b. Indirect: the Von Willebrand factor will stick to the collagen when there's injury and help the platelet to bind to it (عامل وسيط بينهم).

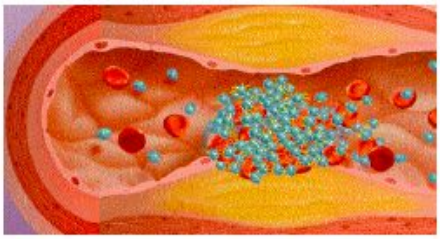
2. Activation





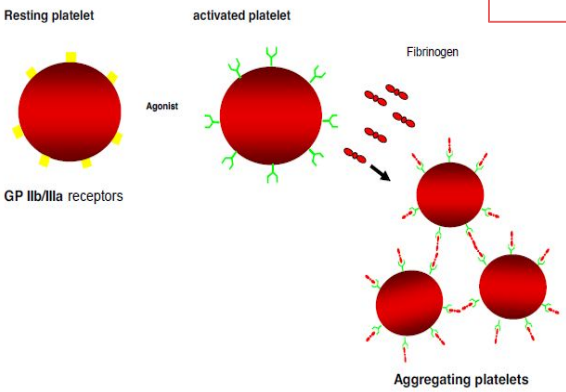


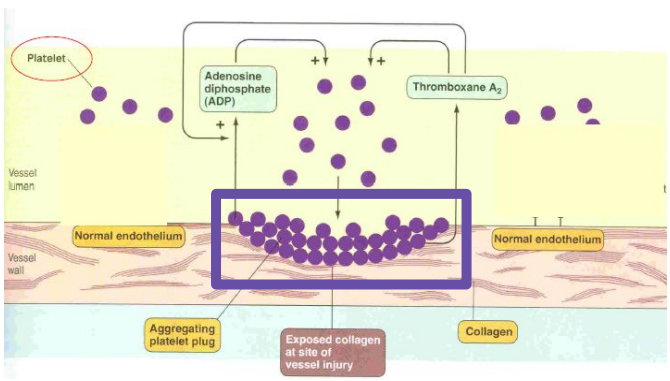
3. Aggregation



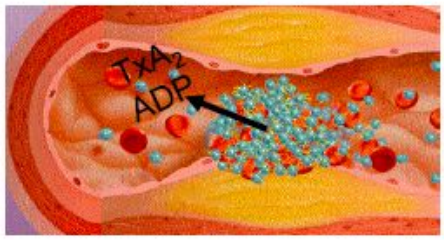
3- Aggregation phase

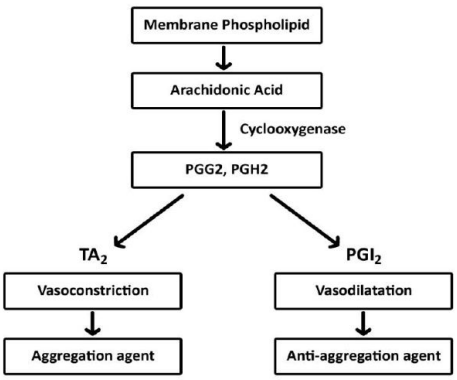
As you can see in the pic below that the activated platelets expose **GP IIb-IIIa receptors in green** why? For binding of fibrinogen in **red** and form the plug , what's the role of fibrinogen in aggregation phase? It is needed to join platelet to each other via platelet fibrinogen receptors.





4. Secretion



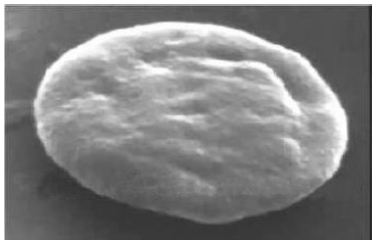


4- Secretion (release reaction phase):

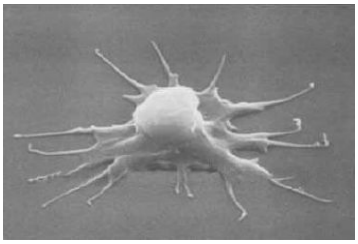
1. ADP strong activator of platelets [makes it sticky]> activate aggregation.
2. 5HT → vasoconstriction.
3. Platelet phospholipid (PF3) → clot formation.
4. Thromboxane A2 (TXA2) is a prostaglandin formed from arachidonic acid. It is an example of +ve feedback
   - Function:
     - Vasoconstriction
       (decreasing blood flow through the injured vessel)
     - Platelet aggregation
       (TXA2 inhibited by aspirin).
       (Aspirin will inhibit cyclooxygenase enzyme)

5. Clot reaction

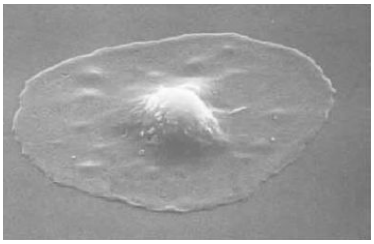
Myosin and actin filaments in platelets are stimulated to contract during aggregation further reinforcing the plug and help release of granule contents



Resting platelets



Activated platelet

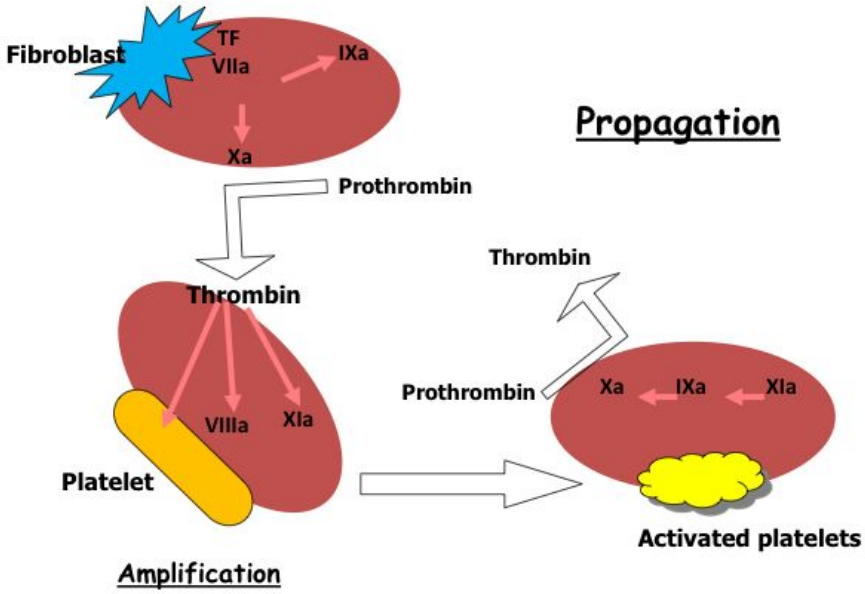


Spread platelet (cover the injured area better)

Platelet haemostatic plug formation

- Platelets activated by adhesion
- Extend projections to make contact with each other
- Release: thromboxane A2, serotonin & ADP >> activating other platelets
- Serotonin & thromboxane A2 are vasoconstrictors decreasing blood flow through the injured vessel.
- ADP causes stickiness and enhances aggregation

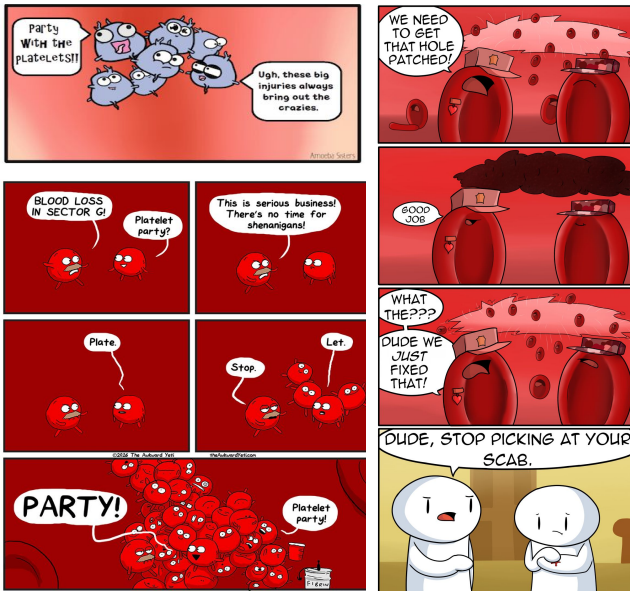
Role of platelet in blood coagulation (The cell based model of blood coagulation)



Don't memorize the coagulation cascade! The only thing you should know is the role of platelets in fibrin formation:

- For coagulation to happen we need cellular contribution, mainly platelets. Hence, the name Cell Based Model.
- When the platelets gets activated, we have the PF3 that is usually inside the platelet. When platelet is activated the PF3 will go to the surface of the platelet (and we mentioned how PF3 is important for clot formation) and this means that the reaction of coagulation happens on the surface of cell membrane. The product of coagulation is thrombin.
- Thrombin will convert fibrinogen (inactive form) to fibrin (active form)

اللي مروق يتخيل نفسه platelet
ويشوف الفيديو, يونس ترا يتزحلقون



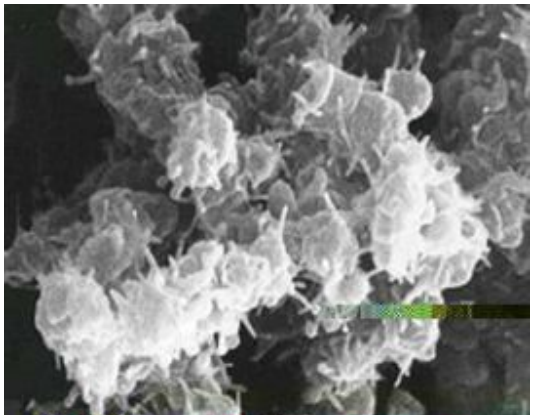
Platelet function

Maintenance of vascular integrity

1

Initial arrest of bleeding by **platelet plug formation**

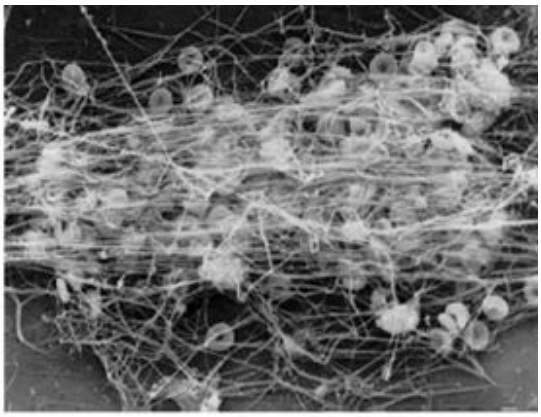
Primary clot weak and unstable



2

Stabilization of hemostatic plug by contributing to fibrin formation

Secondary clot ,stable

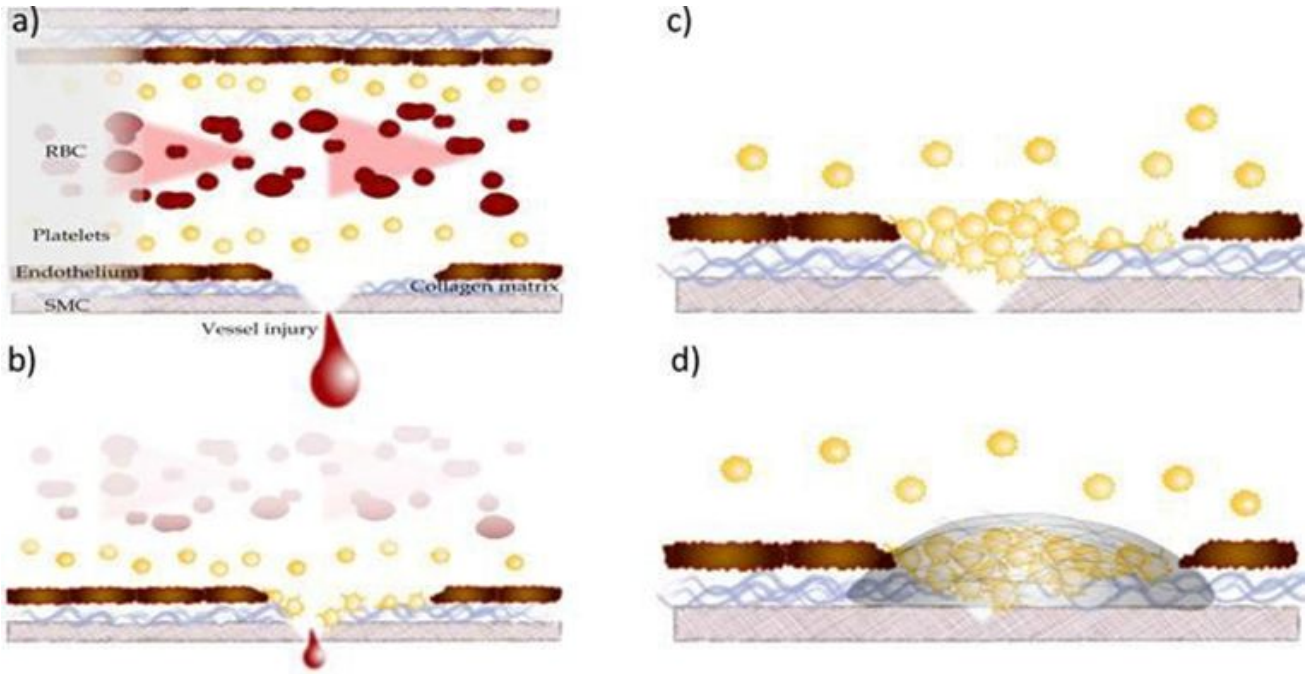


So now we already have the clot but its weak and can cause small blood vessels injuries (primary clot). How do we strengthen or stabilize weak clots? By formation of fibrin

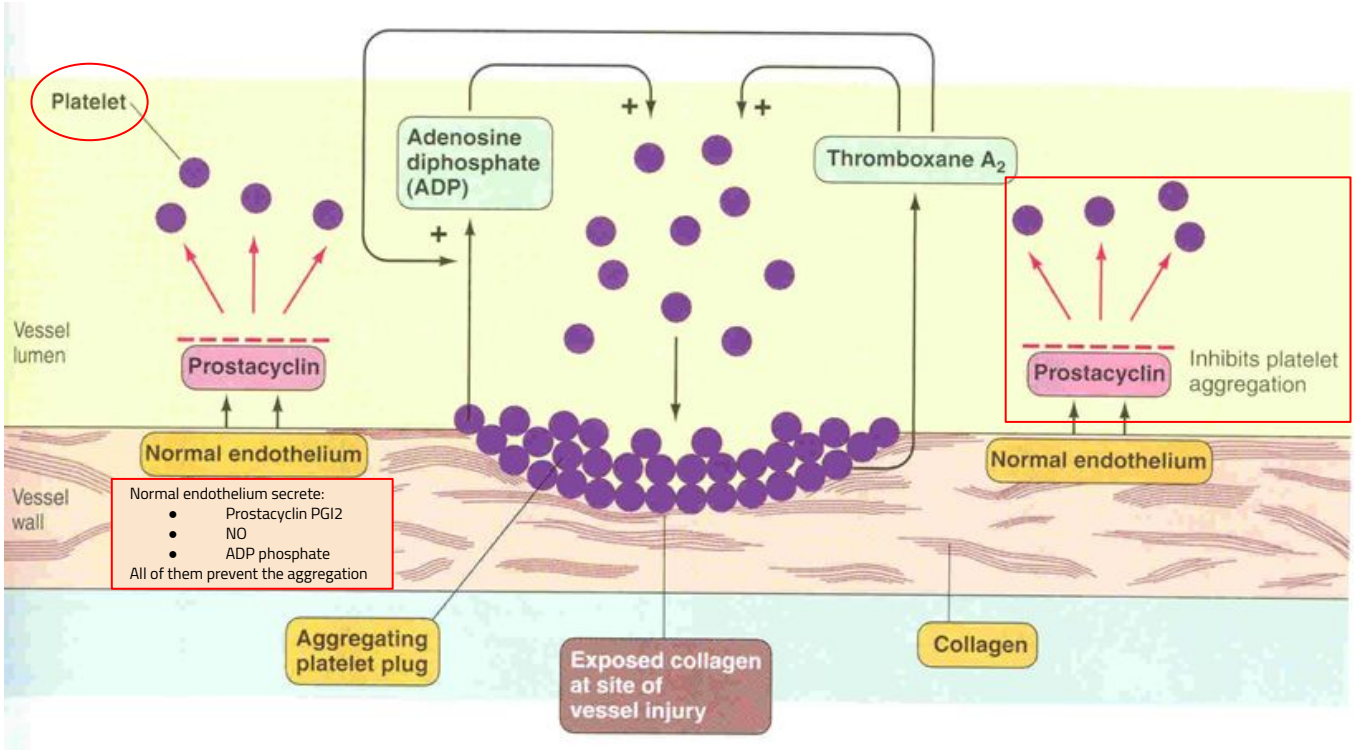
Well imagine the plug as a wall and you stack stones (platelets) to build it and you add the cement (Fibrin) in-between the layers of the rocks to stabilize and strengthen the wall.

➔ An adequate number and function of platelets is essential to participate optimally in hemostasis

Platelet hemostatic plug formation:



Platelet aggregation:

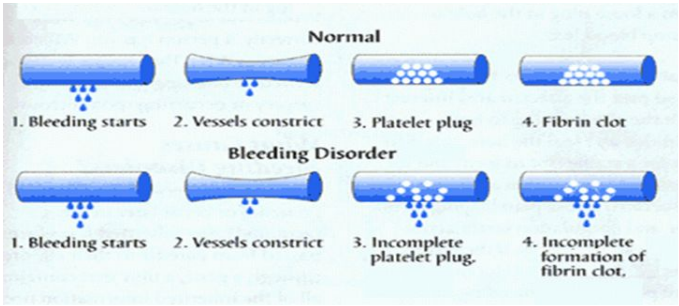


Summary of platelet activation:

- Platelets are activated when brought into contact with collagen exposed when the endothelial blood vessel lining is damaged.
- Activated platelets release a number of different coagulation and platelet activating factors.
- Transport of negatively charged phospholipids to the platelet surface; provide a catalytic surface for coagulation cascade to occur.
- Platelets adhesion receptors (integrins): Platelets adhere to each other via adhesion receptors forming a hemostatic plug with fibrin.
- Myosin and actin filaments in platelets are stimulated to contract during aggregation further reinforcing the plug and help release of granule contents
- GPIIb/IIIa: the most common platelet adhesion receptor for fibrinogen and von Willebrand factor (vWF)

Bleeding disorders:

Normally healthy people always get minor injuries, especially in small blood vessels, but it doesn't show because the platelets are doing their role well.
Signs of platelet dysfunction:
Hemophilia, bruises (easily bruised without trauma), nose bleeding (epistaxis), abnormal menstrual cycle,



Bleeding can result from:

Platelet defects:

- deficiency in number (thrombocytopenia) Congenital disorder
- defect in function Can congenital or acquired
  Acquired platelet disorder? A person taking aspirin

Thrombocytopenia the causes of decreased platelet counts are:

I. Decreased production

- Leukemia or lymphoma
- Cancer treatments such as radiation or chemotherapy
- Various anemias
- Toxic chemicals
- Medications: diuretics, chloramphenicol
- Viruses: chickenpox, mumps, Epstein-Barr, parvovirus, AIDS
- Alcohol in excess
- Genetic conditions: Wiskott-Aldrich, May-Hegglin

II. Increased destruction

Autoimmune diseases: Idiopathic (immune) thrombocytopenic purpura
Medications: quinine, antibiotics containing sulfa, Dilantin®, vancomycin, rifampin, heparin-induced thrombocytopenia
Surgery: man-made heart valves, blood vessel grafts, bypass machines
Infection: septicemia
Pregnancy: about 5% of pregnant women develop mild decrease
Thrombotic thrombocytopenic purpura Disseminated intravascular coagulation

III. Abnormal distribution

Splenomegaly with sequestration in the spleen.

IV. Pseudothrombocytopenia

- Partial clotting of specimen
- EDTA-platelet clumping
- Platelet satellitism around WBCs
- Cold agglutinins
- Giant platelets

Congenital platelet disorders:

Disorders of Adhesion:

- Bernard-Soulier

Disorder of Aggregation:

- Glanzmann thrombasthenia

Disorders of Granules:

- Grey Platelet Syndrome
- Storage Pool deficiency
- Hermansky-Pudlak syndrome
- Chediak-Higashi syndrome

Disorders of Cytoskeleton:

- Wiskott-Aldrich syndrome

Disorders of Primary Secretion:

- Receptor defects (TXA2, collagen ADP, epinephrine)

Disorders of Production:

- Congenital amegakaryocytic thrombocytopenia
- MYH9 related disorders
- Thrombocytopenia with absent radii (TAR)
- Paris-Trousseau/Jacobsen

Factors affecting blood platelet count:

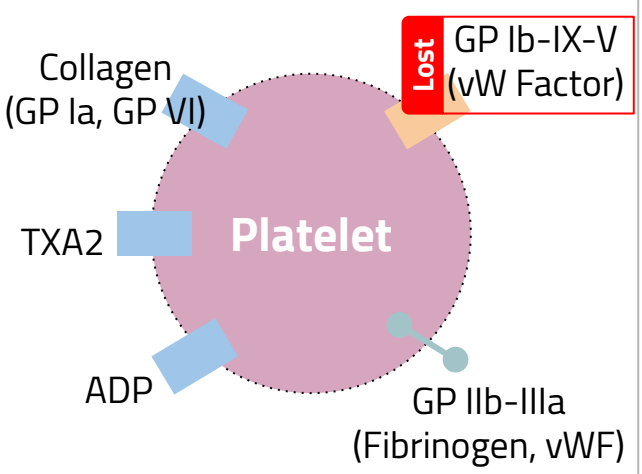
- Age ↓ in newborn
- Menstrual cycle
  - ↓ Prior to menstruation
  - After menstruation ↑
- Pregnancy ↓
- Injury ↑
- Adrenaline ↑
- Hypoxia ↑
- Smoking ↓
- Nutritional deficiencies
  - ↓ eg; Vit. B12, folic acid and iron.

The result of hypersplenism = low platelets count
The result of splenectomy = high platelets count

Platelet Activation

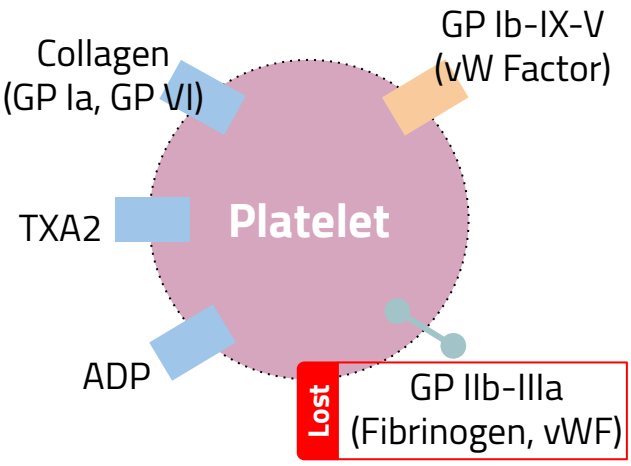
- Adhesion (**Bernard-Soulier Syndrome** "BSS")
- Shape change
- Aggregation (**Glanzmann Thrombasthenia**)
- Release
- Clot Retraction

Bernard-Soulier Syndrome



Deficiency of glycoprotein Ib
It is a giant platelet disorder, meaning that it is characterized by abnormally large platelets.

Glanzmann Thrombasthenia



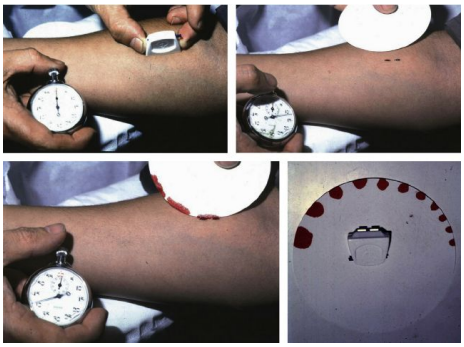
Abnormal platelets. extremely rare coagulopathy. Deficiency or low levels of glycoprotein IIb/IIIa (Gp IIb/IIIa), which is a receptor for fibrinogen. As a result --> no fibrinogen bridging of platelets to other platelets can occur, and the bleeding time is significantly prolonged

How to investigate for a platelet disorder?

Tests of Platelet Functions:

- Bleeding time (Duke method)
- Peripheral smear and Platelet count
- Electron-microscopy
- Platelet Aggregation
- Platelet Function Analyzer (PFA- 100)
- Flow-cytometry
- Granule release products

Bleeding Time



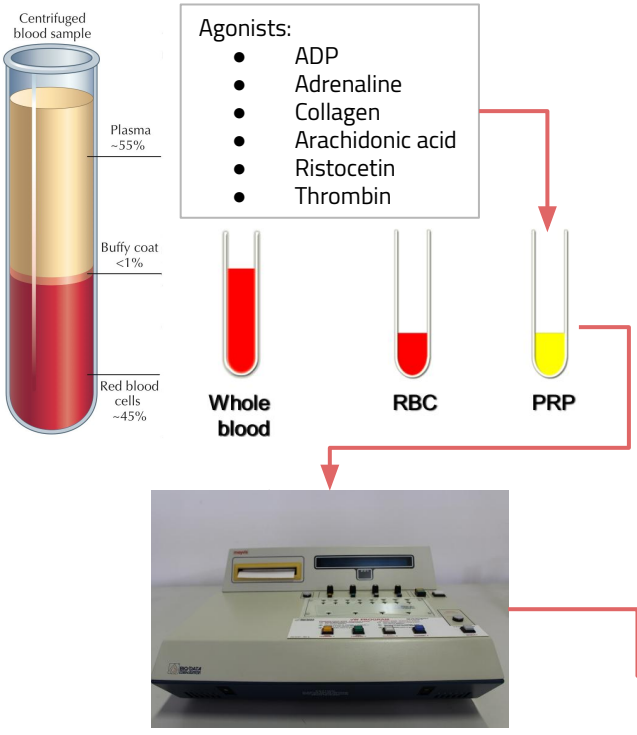
Platelet Function Test

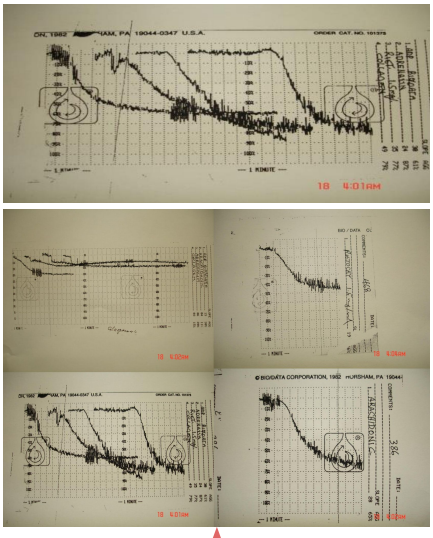
Laboratory Testing of Platelet Functions

Platelet Aggregation in (PRP) Platelet rich plasma:

- Provides information on time course of platelet activation.
- Agonists:
  - ADP
  - Adrenaline
  - Collagen
  - Arachidonic acid
  - Ristocetin
  - Thrombin

Reference ranges need to be determined for each agonist.





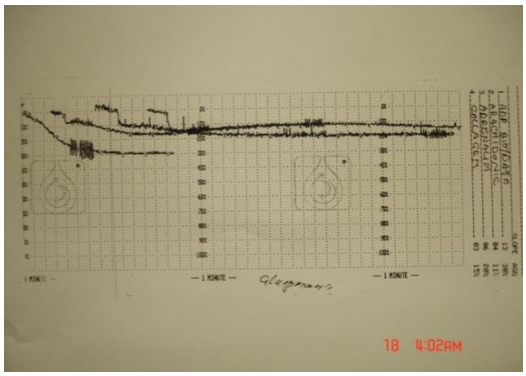
Summary

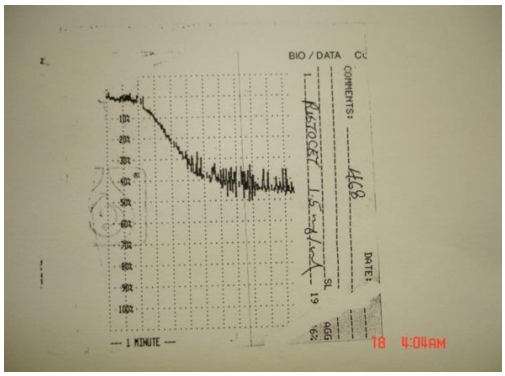
- platelets are cell fragments derived from megakaryocyte in the bone marrow and its regulated by **thrombopoietin**, how? By:
  a. increasing the number of megakaryocytes.
  b. increasing the number of cells produced by megakaryocyte.
- Platelets play a pivotal role in haemostasis by arresting bleeding from an injured blood vessels.
- Bleeding can result from: Platelet defects (acquired or congenital).
- Platelet function tests are used to detect abnormal platelet function.

Case Study

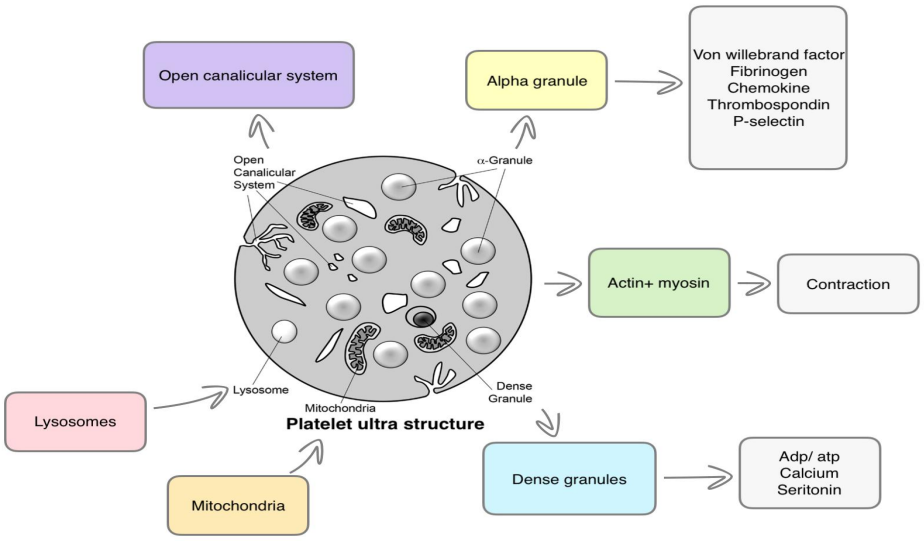
A 7 years old girl complaining of:

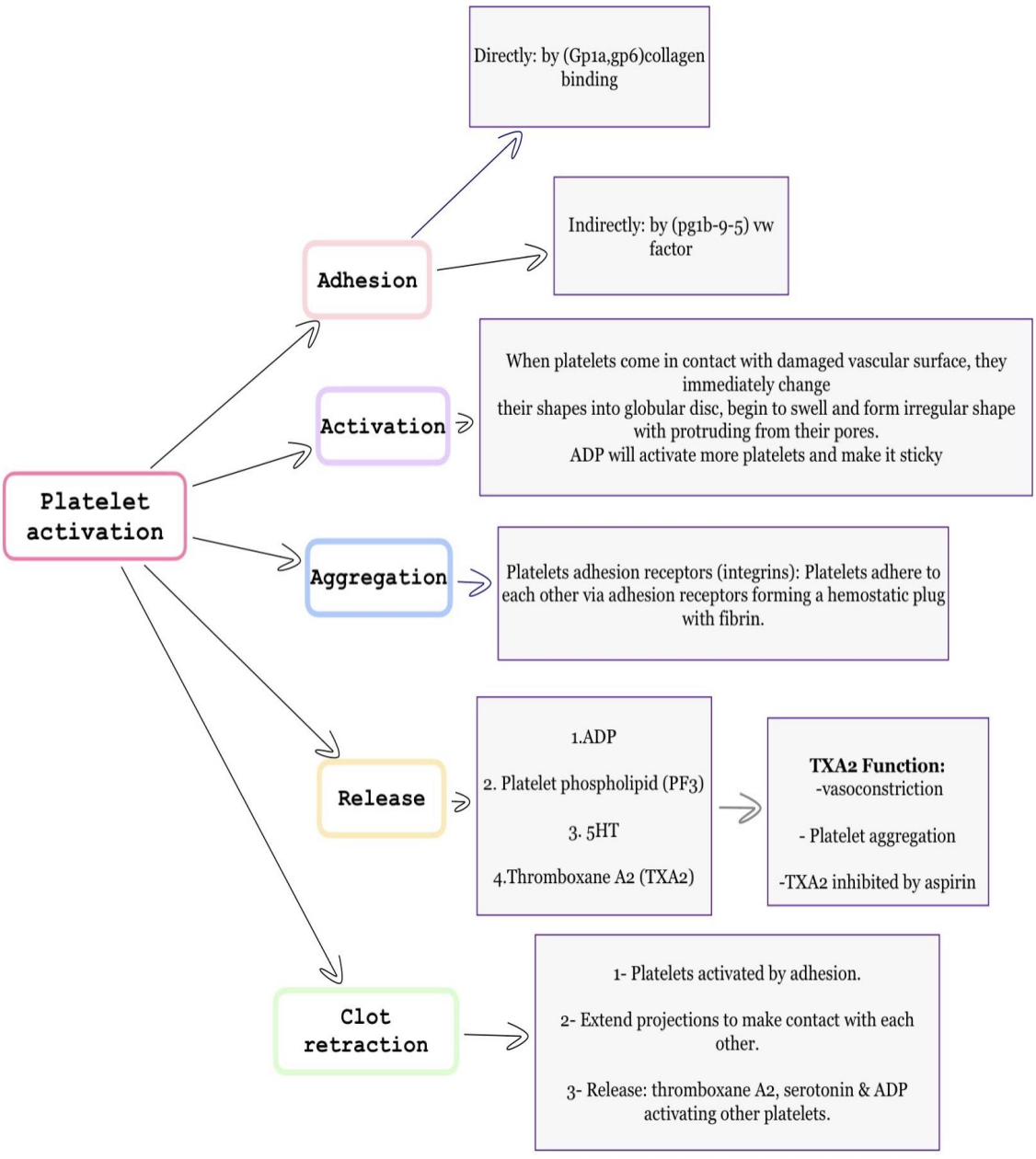
- Severe bruising since birth and if she had injury she would bleed for days.
- She had epistaxis which lasted for days.
- Her mother said "she just bruise more easily than her older sister"
- **Investigation**
  - CBC: RBC, WBC, Platelets
  - Platelet morphology: Normal
  - Aggregometry: Absent platelet aggregation in response to ADP, collagen, thrombin, & epinephrine.
- **Diagnosis**
  - Glanzmann's Thrombasthenia





Summary


Open canalicular system
Alpha granule
Von willebrand factor
Fibrinogen
Chemokine
Thrombospondin
P-selectin
Open Canalicular System
α-Granule
Actin+ myosin
Contraction
Lysosomes
Lysosome
Mitochondria
Dense Granule
Platelet ultra structure
Mitochondria
Dense granules
Adp/ atp
Calcium
Seritonin


Platelet activation
Adhesion
Directly: by (Gp1a,gp6)collagen binding
Indirectly: by (pg1b-9-5) vw factor
Activation
When platelets come in contact with damaged vascular surface, they immediately change their shapes into globular disc, begin to swell and form irregular shape with protruding from their pores.
ADP will activate more platelets and make it sticky
Aggregation
Platelets adhesion receptors (integrins): Platelets adhere to each other via adhesion receptors forming a hemostatic plug with fibrin.
Release
1.ADP
2. Platelet phospholipid (PF3)
3. 5HT
4.Thromboxane A2 (TXA2)
TXA2 Function:
-vasoconstriction
- Platelet aggregation
-TXA2 inhibited by aspirin
Clot retraction
1- Platelets activated by adhesion.
2- Extend projections to make contact with each other.
3- Release: thromboxane A2, serotonin & ADP activating other platelets.

MCQs

Q1: Which one would be inhibited by Aspirin to prevent clot formation?

A. ADP
B. Thromboxane A2
C. Serotonin
D. PGI

Q2: Bernard Soulier Syndrome is caused by:

A. A disorder of granules
B. A disorder of cytokines
C. A disorder of aggregation
D. A disorder adhesion

Q3. ADP/ATP can be found in: :

A. Dense granules
B. OCS
C. Alpha granules
D. Mitochondria

Q4. Low platelet count can be caused by:

A. Hypersplenism
B. Splenomegaly
C. Hepatomegaly
D. A&B

Q5. The coagulation pathway that begins with tissue thromboplastin is:

A. Intrinsic pathway
B. Extrinsic pathway
C. Common pathway
D. Fibrin stabilization

Q6. Why do some malnourished patients bleed excessively when injured?

A. Vitamin K deficiency
B. Platelet sequestration by fatty liver
C. Serum bilirubin raises neutralizing thrombin
D. Low serum- protein levels cause factor XIII problems

Q7. Which one of the following would best explain a prolonged bleeding time tests?

A. Hemophilia A
B. Hemophilia B
C. Thrombocytopenia
D. Coumarin use

Q8. A teenage boy with numerous nosebleeds was referred to a physician for evaluation prior to a minor surgery. His prothrombin time(PT) was 11 secs (11-15sec normal), partial thromboplastin time(PTT) was 58 secs (25-40sec normal), and bleeding time was 6.5 min (2-7 min normal). Which of the following is most likely abnormal in this young man?

A. Intrinsic pathway
B. Extrinsic pathway
C. Decreased platelet number
D. Defective platelet

Answers
1: B
2: D
3: A
4: D
5: B
6: A
7: C
8: A