Platelets

They are not cells but small fragments of megakaryocyte cytoplasm. Platelets are about 2-4 µm in diameter and possess lysosomes, endoplasmic reticulum, a Golgi complex and Golgi vesicles or granules that contain a variety of factors involved in platelet function. Platelets have pseudopods and are capable of amoeboid movement and phagocytosis. The platelet count for normal individuals ranges from 130000 - 400000 platelets/1mm^3 (average about 250000/ 1mm^3) of blood.

Function of platelets:

1. They secrete growth factors that stimulate mitosis in fibroblasts and smooth muscles, and help to maintain the lining of blood vessels.
2. They secrete vasoconstrictors that cause vascular spasm in injured vessels.
3. They form temporary platelet plugs to stop bleeding.
4. They are capable of phagocytosis and destroy bacteria
5. They secrete chemicals that attract neutrophils and monocytes to sites of inflammation.
6. They dissolve blood clots that have out-lasted their usefulness.

Hemostasis:

It is a process of stopping bleeding (preventing blood loss). It includes:-

1. vascular spasm
2. platelets plug formation
3. blood clot formation (coagulation)
4. clot retraction
5. clot dissolution
6. Growth of fibrous tissue into the blood clot to close the whole vessel permanently.

Platelets play an important role in all above processes.

1. **Vascular Spasm:**

   When a blood vessel has been cut or ruptured (damaged), vascular contraction (spasm) occurs, this contraction increases the resistance to blood flow that minimize blood flow and reduces the loss of blood from ruptured vessel. **The following mechanisms contribute in vessel contraction: -**

   - Local myogenic contraction of the blood vessel due to direct damage to the vessel wall
   - Local nervous reflexes
   - Local humoral factors from the traumatized tissue
   - Platelets from the smaller vessels are responsible for most of the vascular constriction by releasing vasoconstriction substances like serotonin, epinephrine and thromboxane A2

The local vascular spasm can last for many minutes and maintained long enough, during which time, the processes of platelet plugging and blood clotting can take place. People who lack the normal number of platelets tend to develop numerous small hemorrhages in their skin and internal organs.

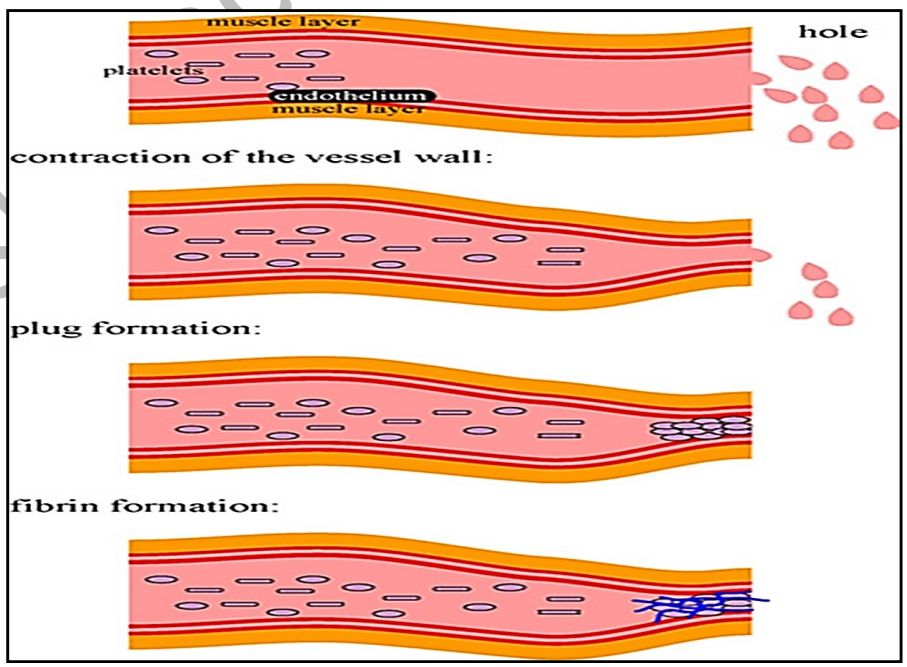

Figure (1): The Vascular Spasm Phase of the Hemostasis

2. Platelet Plug Formation:

This process can be described as series of steps, many of these events occur simultaneously:-

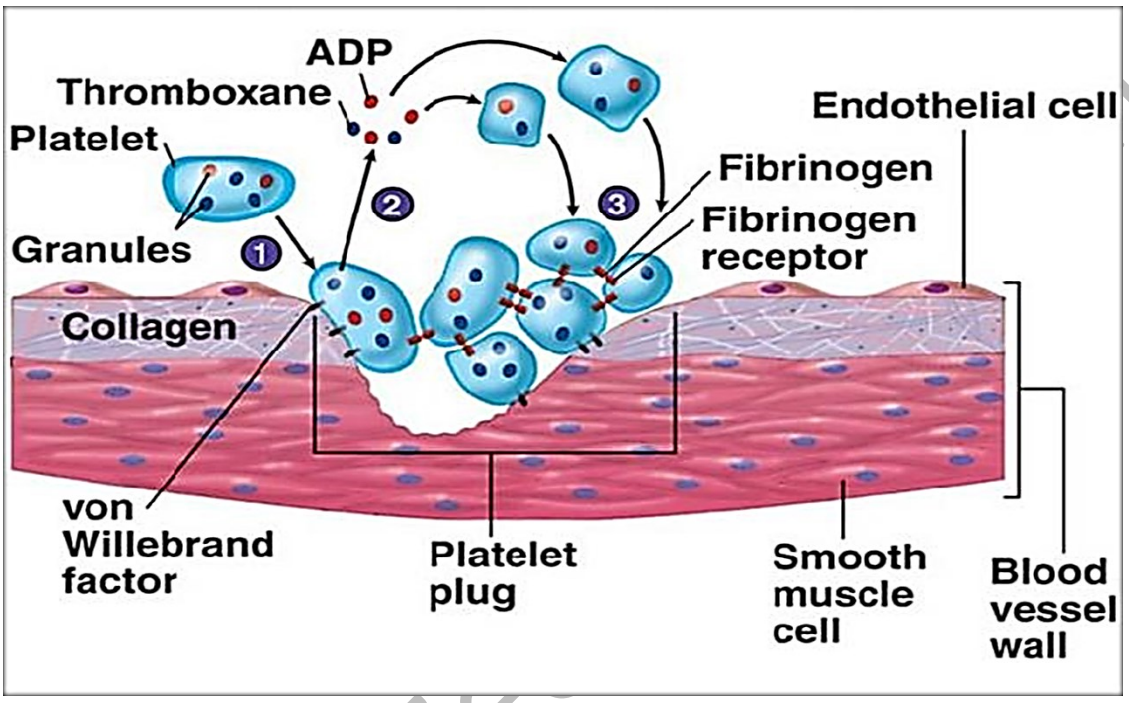

Figure (2): Aggregation of Platelets at Site of Injury and Formation of Platelet Plug

a. Platelets Adhesion:

This step involves the binding of platelets to the exposed collagen of the damaged vessel through a key protein which is Von Willibrand factor (VWF). This protein is secreted by megakaryocytes, platelets and endothelial cells lining the blood vessel. VWF binds to platelets surface receptors and collagen, causing the platelets to adhere to collagen while other platelets can bind directly to the collagen.

b. Platelets Activation:

Platelets adhere to collagen by VWF become activated. This activation stimulates the platelets to secrete certain substances like Adenosine diphosphate (ADP) and thromboxane which will result in

further activation of other platelets. Also, activated platelets secrete serotonin and epinephrine which cause vasoconstriction.

c. Platelets Aggregation:

As platelets become activated, ADP stimulates morphological changes in the platelets causing them to express surface receptors that can bind to fibrinogen (a plasma protein). In platelets aggregation, the fibrinogen forms a bridge between the surface receptors of different platelets resulting in the formation of platelets plug. ADP stimulates the production of thromboxane A2 (TX_{A2}) which supports further platelets aggregation.

d. Activated platelets express phospholipids (platelet factor III) and coagulation factor V which are important part of clot formation.

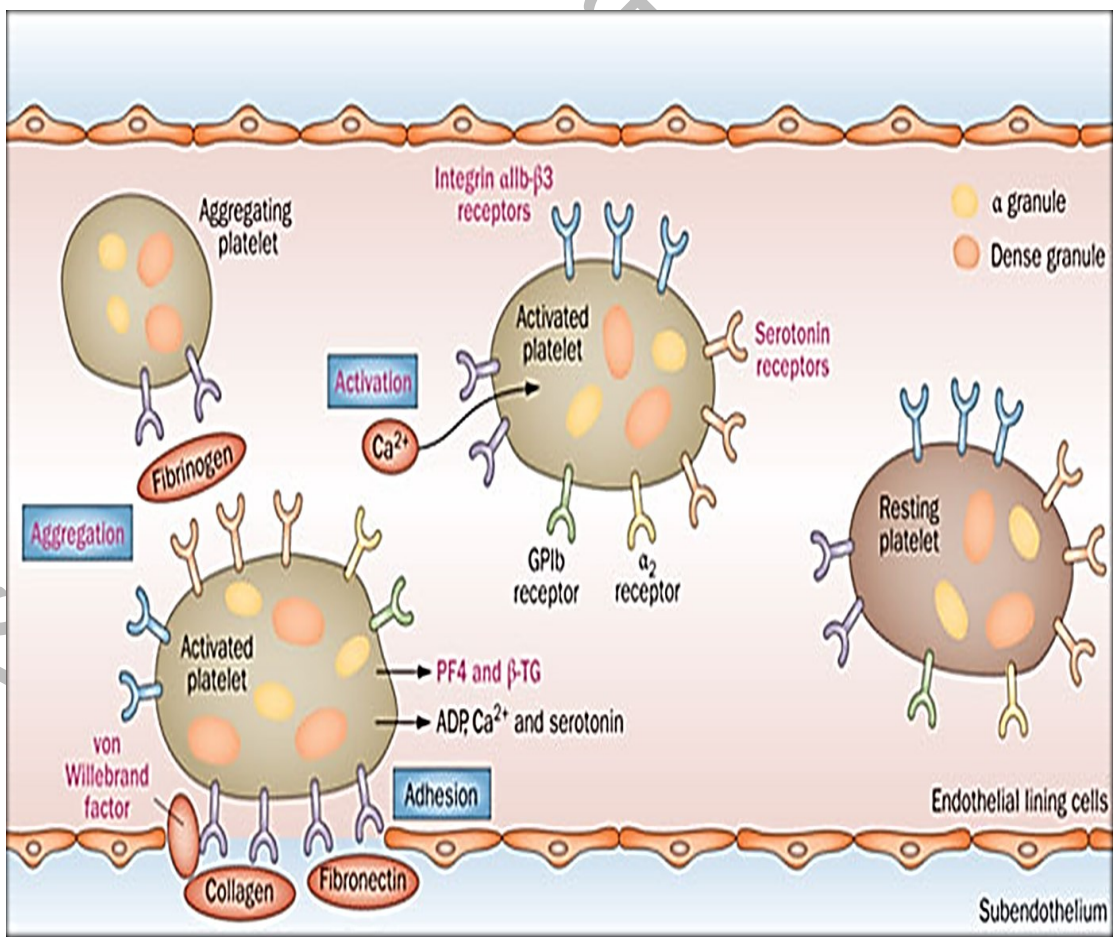

Figure (2): The Process of Platelet Plug Formation

3. **Clot Formation (Coagulation):**
   Vascular spasm and platelets plug alone are not sufficient to close large cuts. **A blood clot is a network of threadlike protein fibers called fibrin that traps blood cells, platelets and fluid.** Formation of blood clot depends on a number of plasma proteins called coagulation factors, which normally are present in inactive form and do not cause clotting. **There are 2 separate pathways for coagulation:**
   a. **Intrinsic pathway:** begins with chemicals that are inside the blood. It starts when plasma factor XII is activated by coming into contact with the collagen layer of a damaged blood vessel.
   b. **Extrinsic pathway:** begins with chemicals that are outside the blood. It starts with tissue factor (thromboplastin) which is released from damaged tissues (outside plasma).

Control of clot formation:-

Without control, coagulation would spread from the point of initiation to the entire circulatory system. **To prevent unwanted clotting, the blood contain several anticoagulants like anti-thrombin (produced by the liver), and heparin (produced by basophiles). Heparin counteracts the effect of thrombin by causing vasodilation and by inhibiting the release of coagulation factor from platelets.**

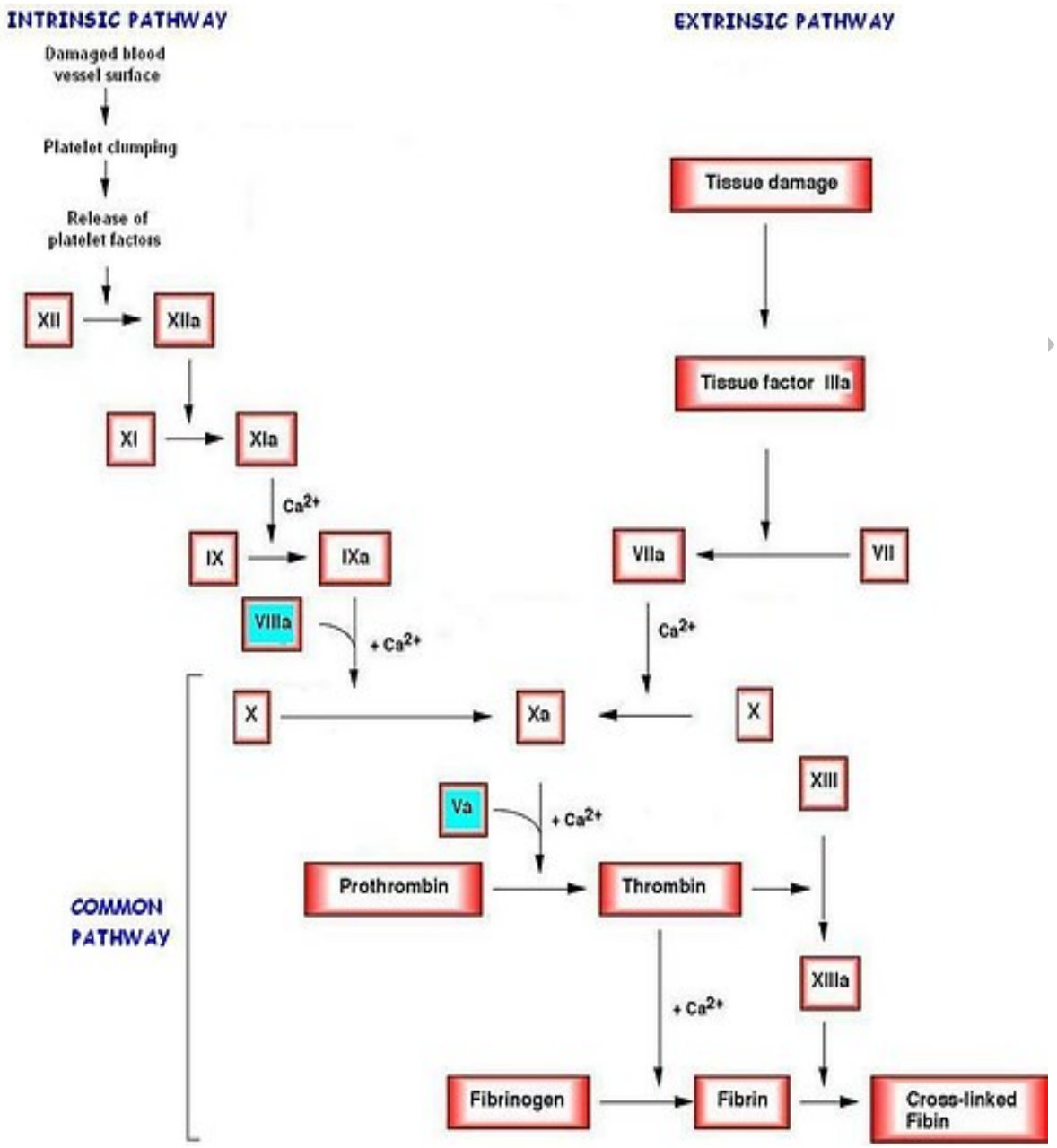

Figure (4): Intrinsic and Extrinsic Pathways of Coagulation

4. Clot Retraction:

Clot retraction normally occurs within 20-60 minutes after a clot has formed contribution to hemostasis by squeezing serum from the clot and joining the edges of the broken vessel. Adequate numbers of platelets are necessary for clot retraction to occur.

Factors hastening clotting: -

1. Contact with foreign body: the application of gauze aids very considerably in the speedy formation of a clot and arrest of hemorrhage.
2. temperature slightly higher than that of body: so, during surgical operations, bleeding surfaces are packed with swabs soaked in hot saline at 49 C˚

Factors retarding clotting:-

1. Addition of sodium or potassium citrate: when a blood from a donor is taken directly into a solution of potassium citrate 3.8%, it may be kept for periods without risk of clotting.
2. Local cold

Determination of Bleeding Time & Clotting Time

1. Bleeding Time:

Is the time it takes to stop bleeding, it measures the primary phase of hemostasis; the interaction of platelets with the blood vessel wall and the formation of a hemostatic plug.

The normal value for bleeding time is 1- 5 min. **Duration of bleeding time depends on quantity and quality of platelets and ability of blood vessels to constrict.**

Objective (Aim):

It is done by Duck´s method. It is of great value in detecting vascular abnormality, platelets abnormalities and deficiencies.

Procedure:

- Clean the tip of your finger with 70% alcohol, and then dry it with a piece of cotton.
- Puncture the finger with sterile lancet and record the time.
- At 15 sec intervals wipe the blood drop away completely with filter paper.
- Continue this procedure until no more blood stains appear on the filter paper.
- Count the number of blood spots and divide it on 4 to obtain bleeding time in minutes.

2. **Clotting Time:**

It is the time required to form a fibrin clot from the beginning of shedding of blood. In the normal case, clotting time is 5-12 min.

Objective (Aim):

It is done by Wright method. This test is of value because it measures the time required to form a stable fibrin clot and to detect the amount of thromboplastin formed

Procedure:

- clean the tip of your finger with 70% alcohol, and then dry it with a piece of cotton
- puncture the finger with sterile lancet and obtain large drop of blood (note the time when the drop appears)
- Rapidly draw blood into a non-heparinized capillary by holding the tube in the drop of the blood in a horizontal position. Allow blood to rise at least half the tube
- Wait 3 min from the starting of filling and break of a small piece of the capillary tube. Repeat every 30 sec until you notice that the blood has clotted (clotting has occurred when a fine fibrin thread is visible between the two pieces of tubing)
- Note the time when clotting first is seen. Divide the number of pieces on 2 to obtain clotting time in min
- do not forget the addition of waiting time to the results

Home Work:

1. Why clotting time is more than bleeding time?
2. In clotting time experiment. Why did you use (blue) capillary tube rather than (red) capillary tube?
3. In bleeding time experiment. After puncturing your finger, why it should be gently touched to the filter paper and not pressed?
4. Show by figure the difference between the intrinsic and extrinsic pathways of coagulation?