University of Al-Ameed
College of Pharmacy

Physiology

Determination of Bleeding Time And Clotting Tim

Hemostasis

The term hemostasis (Greek Hema = blood; stasis= halt) refers to the cessation of blood loss from a damaged vessel following an injury. Hemostasis can be organized into three major separate but interrelated events (Figure 1): A. Vascular spasm. B. Primary hemostasis (platelets plug formation). C. Secondary hemostasis or Clot formation (Coagulation cascade).

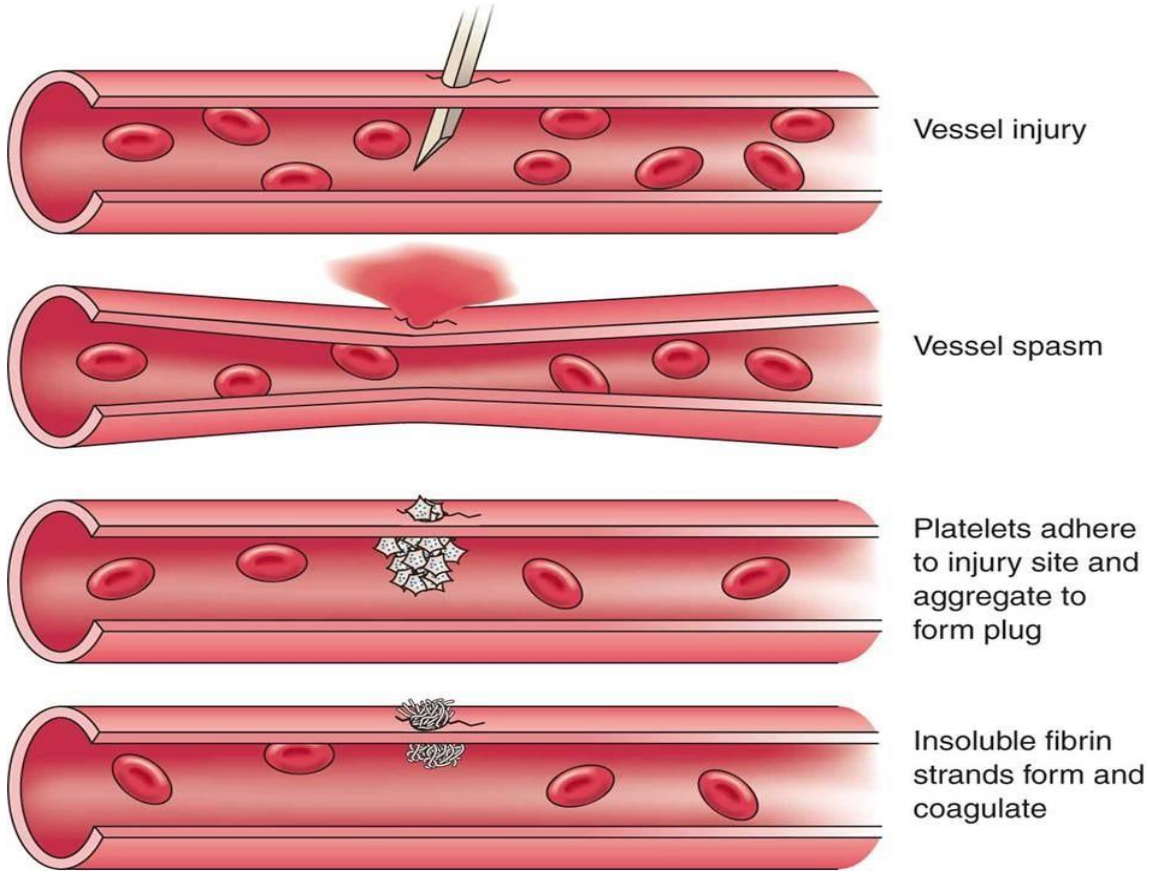


Figure 1: Major components of Hemostasis

A. Vascular spasm (Local vasoconstriction, narrowing of blood vessels):

It is the first reaction to injury in blood vessel (immediate and brief reflex). In this step, a spasm constricts the vessel (Figure 2) and reduces blood flow from the ruptured vessel immediately.

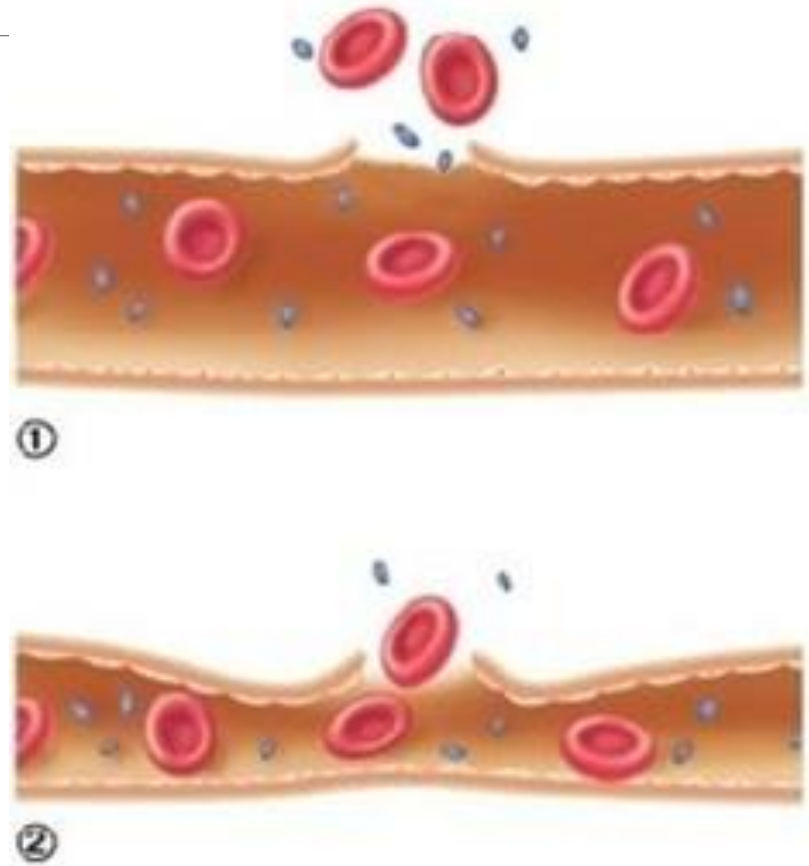


Figure 2: Illustration of vascular spasm

Vasoconstriction occurs due to local spasm (contraction) of the smooth muscle fibers in the walls of injured vessels (sympathetic reflex). The spasm can be maintained by platelet vasoconstrictors. Platelets are responsible for much of the vasoconstriction by releasing thromboxane A2 (TXA2, a potent vasoconstrictor). The spasm can last for minuets or even hours, during this time, the events of platelet plug formation and blood coagulation can start.

B. Primary hemostasis (platelets plug formation):

- The second essential step in hemostasis is the formation of a temporary
- loose platelet aggregate at the area of vascular injury. It involves the adhesion, activation, and aggregation of platelets into a plug as follows (Figure 3):

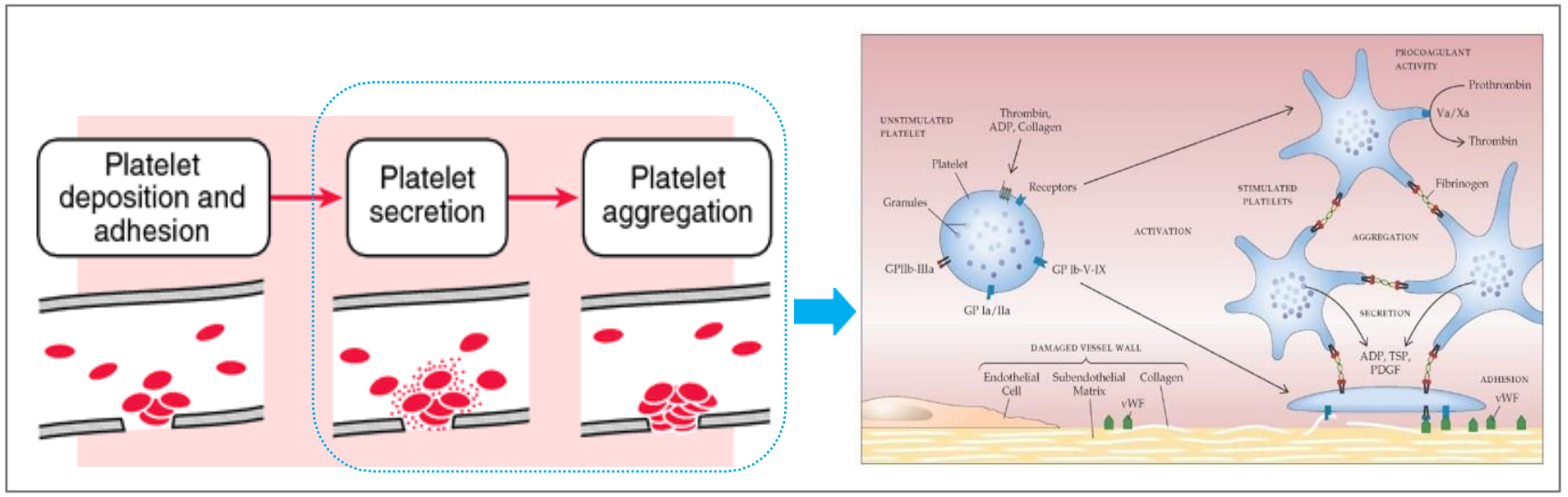


Figure 3: Platelet plug formation.

1. The vessel wall is injured, this leads to collagen exposure.
2. Injured blood vessel releases adenosine diphosphate (ADP), which attracts platelets.
3. Platelets contact with injured wall especially with collagen.
4. Platelets will change their characteristics dramatically (Figure 4):

- They swell.
- Become irregular in shape.
- Produce many protrudes on their surfaces.
- They contract & release their active products.
- They become sticky.

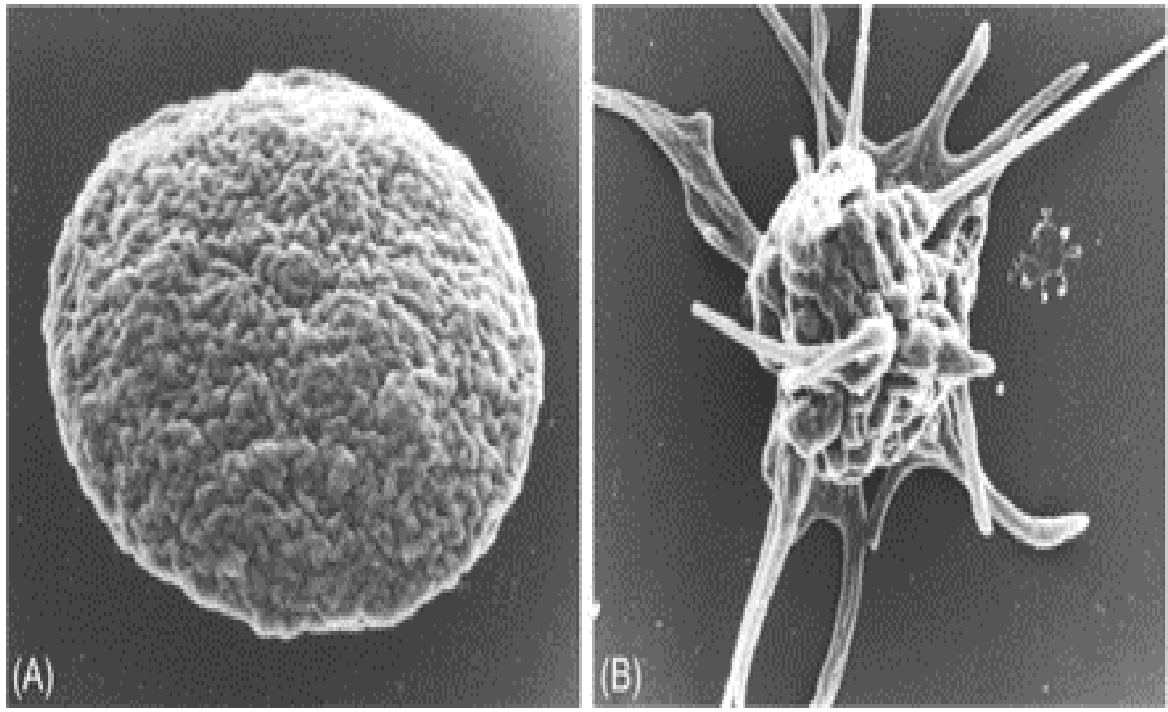


Figure 4: Resting (A) & activated (B) platelets.

5. Activated platelets adhere to collage and a protein called von Willebrand factor (vWF). vWF is secreted from endothelial cells and platelets.

Note: Congenital absence of vWF causes bleeding disorder.

6.They secrete serotonin, ADP, and TXA2.

7. Serotonin, ADP and TXA2 in turn will accelerate vasoconstriction and activate more platelet, increasing the stickiness of the platelets further, so more platelets adhere to the site of injury forming the platelet plug that serves as a barrier against blood flow.

C. Secondary hemostasis or Clot formation (Coagulation cascade):

In the formation of the more stable fibrin clot. Blood clotting can be induced by two pathways: extrinsic and intrinsic pathways, both lead to the conversion of soluble fibrinogen into insoluble fibrin (by an enzyme called thrombin). Fibrin then aggregates to form a meshlike network (clot) at the site of vascular damage (Figure 5). Once the clot is formed, it plugs the ruptured area of the blood vessel and stops further loss of blood.

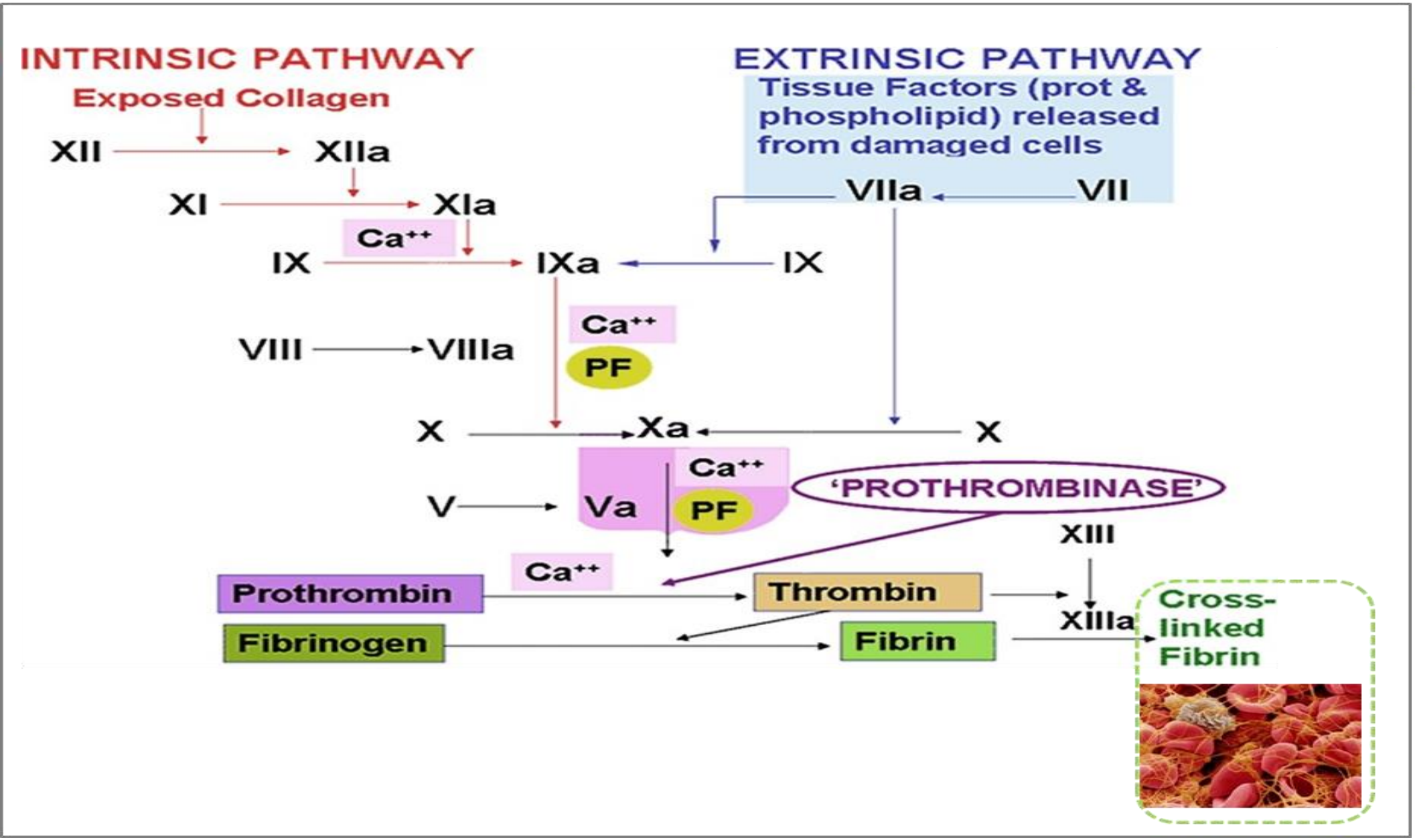


Figure 5: Coagulation cascade.

Coagulation takes place in three essential phases:

❖**Coagulation Phase 1**:Initiated by either the intrinsic or extrinsic pathway. Each pathway cascades toward factor X (Common pathway), and involves a complex cascade of chemical reactions takes place in the blood (dozen of coagulation factors) leading to the formation of a complex of activated substances collectively called (***prothrombin activator or prothrombinase)***.

a. **Extrinsic pathway for coagulation:** Begins with chemicals that are outside the blood (Figure 5):

- It starts with tissue factor (TF, also called thromboplastin) which is released from damaged tissue.
- TF activates factor VII to factor VIIa.
- TF interacts with factor VIIa and Ca^{+2} to form (TF/VIIa complex).
- TF/VIIa complex will activate Stuart factor (Factor X).

b. **Intrinsic pathway for coagulation:** Begins with chemicals that are inside the blood. Exposed collagen activates Factor XII to Factor XIIa, which ultimately lead to activation of Stuart factor (Factor X) through a complex called (IXa/ VIIIa complex), as shown in (Figure 5).

❖**Coagulation Phase 2** (Pathway to Thrombin):Prothrombin activator (also called prothrombinase complex= Factor Xa + Factor Va + Ca^{+2} ions) catalyzes the transformation of prothrombin (Factor II) to the active enzyme (thrombin), (Figure 5). Prothrombin is an inactive precursor of the enzyme thrombin.

❖**Coagulation Phase 3** (Common Pathway):Thrombin converts fibrinogen (Factor I, a plasma protein produced by the liver) into fibrin fibers that entangle the formed elements of the blood to form a gel-like clot (Figure 5). Thrombin also activates fibrin-stabilizing factor (Factor XIIIa), which in the presence of Ca^{+2}, stabilizes the fibrin polymer through covalent bonding of fibrin monomers.

Clot Retraction and Clot Dissolution (Fibrinolysis):

Clot retraction normally occurs within 20-60 mins after a clot has formed, contributing to hemostasis by contraction of the fibrin mesh and squeezing fluid from the clot, thus reducing the size of the clot. During this process, the edges of the endothelium at the point of injury are slowly pulled together again to repair the damage (Figure 6).

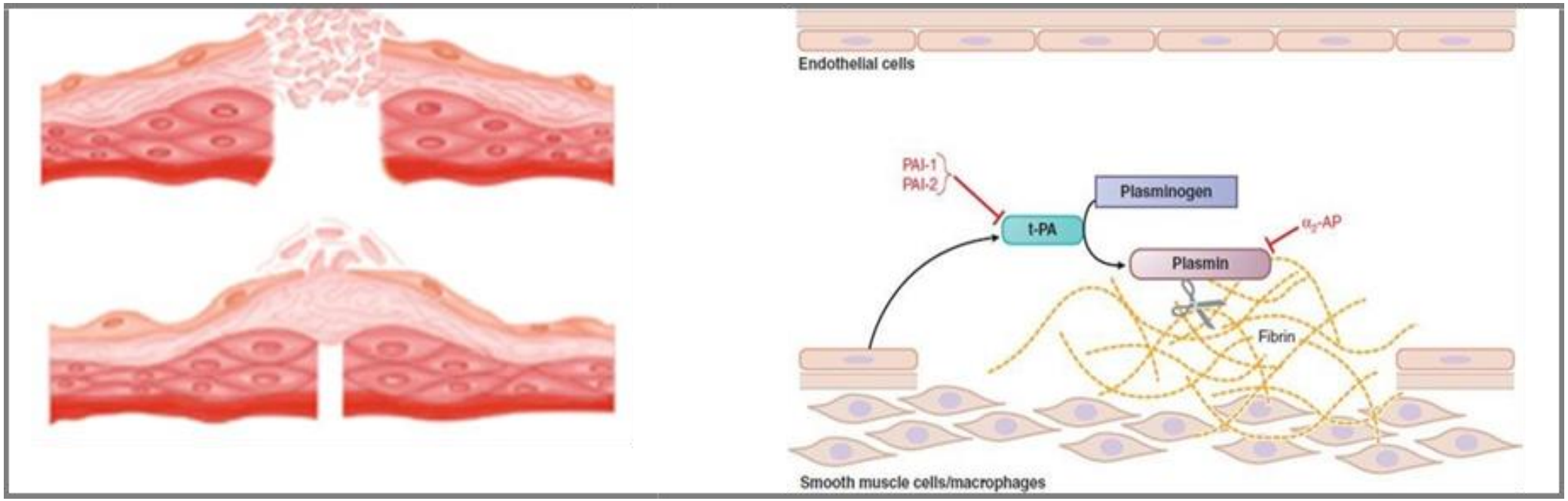


Figure 6: Clot retraction (left side) and fibrinolysis (right side).

Tests for Hemostasis

- Bleeding time.
- Clotting time.
- Platelet count.
- Clot retraction time.
- Clot lysis time.
- Prothrombin time.
- Partial thromboplastin test
- Activated partial thromboplastin time (APTT)

Clinical Applications of Hemostatic Tes

- Screens patients who may possess hemostatic defects (acquired or inherited) that result in excessive clotting or bleeding, such as: thrombophilia (excessive clotting) and hemophilia (inability to clot).
- The bleeding time and clotting time tests are utilized routinely before every minor and major surgery (e.g., tooth extraction), and before and during anticoagulant therapy, whether or not there is a history of bleeding.

Bleeding Time

- **Bleeding Time (BT)-**It is defined as the time taken for the
- stoppage of bleeding from the puncture of the blood vessels.. The normal value of bleeding time is **2-5 minutes**.

Bleeding Time

Bleeding time (Duke Method).

Apparatus and Reagents •

. Alcohol

. Sterile disposable lancet

.Stopwach

. Filterpaper

. Glass slide

Procedure:

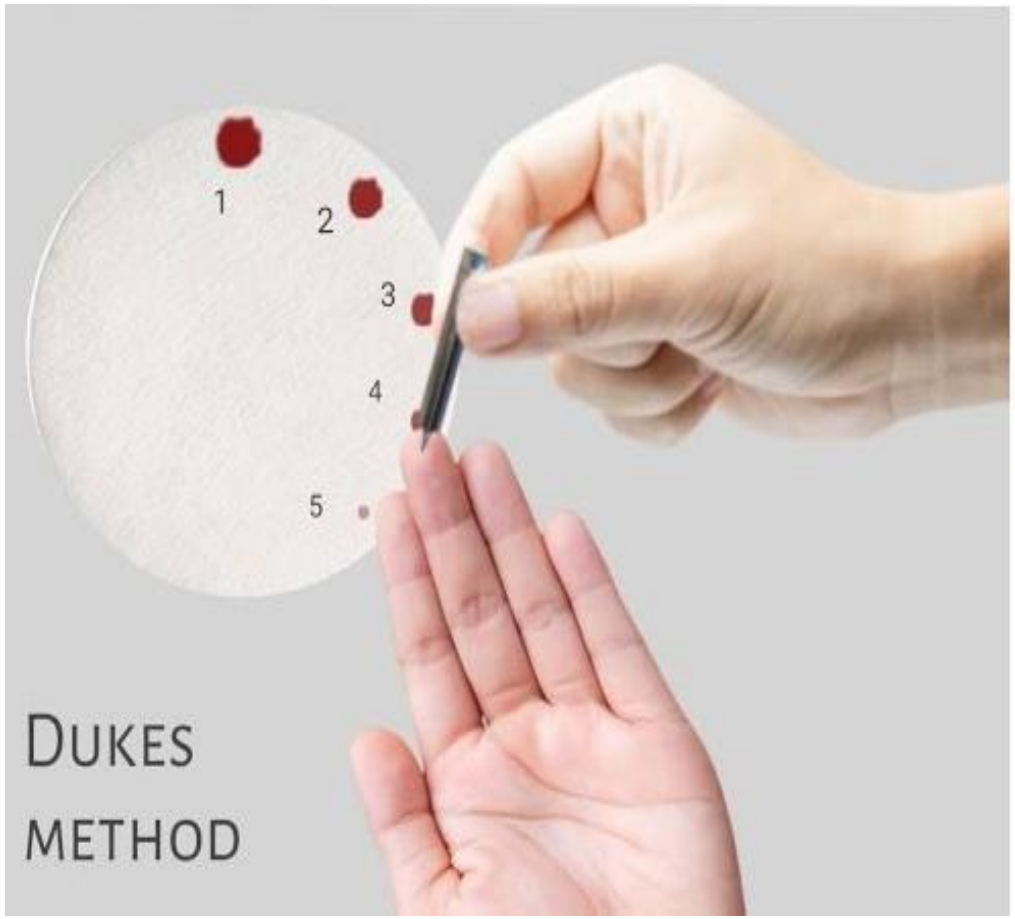
1. Review the Safety Alert in Lab 1.

2.Follow steps 1 through 9 of the “Blood Collection” section of Lab 2 to make a prick with a lancet (preferably on the earlobe or fingertip) and start the stopwatch.

Note: The puncture should be of standard depth (3–4 mm) to obtain reproducible results.

3. With a filter paper the blood is gently blotted every 30 seconds (by placing the edge of filter paper on the top of blood drop without pressing or squeezing the wound). The successive blots become smaller (Figure 7). This procedure is repeated until no blot appears on the filter paper.

4. Note the time when the bleeding stops (blood stain no longer appears on the filter paper) and stop the stopwatch then.



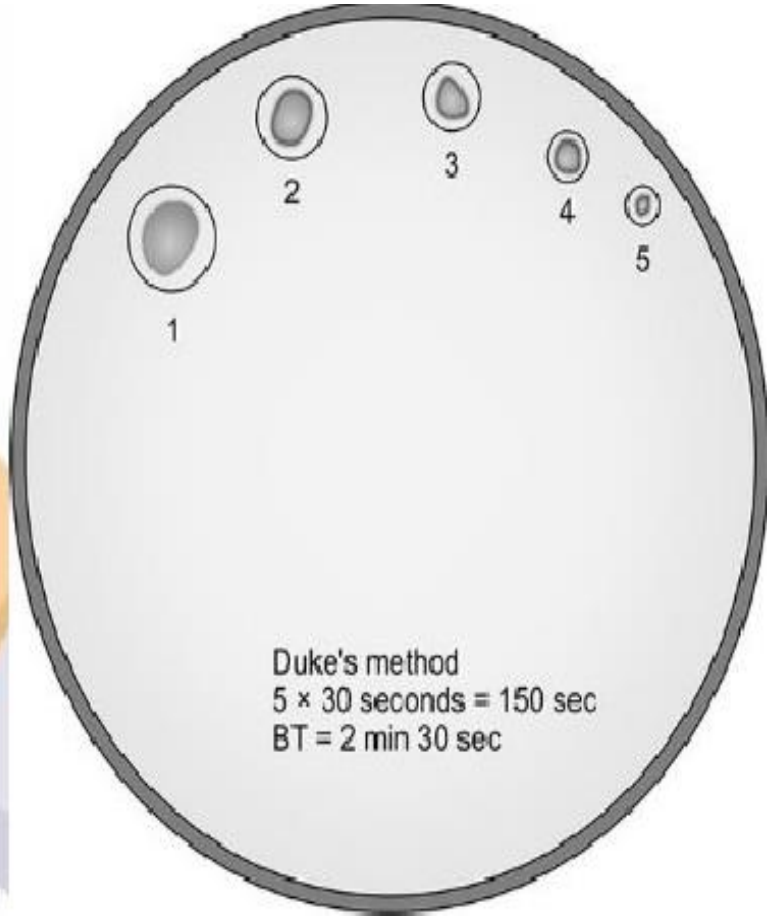


Figure 7: Determination of bleeding time (Duke's method).

Procedure:

5. The time from pricking the finger or earlobe to the stoppage of bleeding is the bleeding time.
6. Count the number of blood spots on the filter paper and divide it by 2, that will give you the bleeding time (in minutes). Express your result in minutes and seconds.
7. Report the results.
8. Dispose of all used materials as described in Lab 1 & 2.

Prolonged bleeding time indicates:

- A vascular (blood vessel) defect.
- Platelet's function defect.
- Thrombocytopenia (low platelets' count).
- Von Willebrand disease (vWD).
- Drugs as dextran, indomethacin, and salicylates (including aspirin) may prolong bleeding time.

Clotting Time

Clotting Time (CT)-It is defined as the time taken for the formation of fibrin clots from the puncture of the blood vessels.

. The normal value of clotting time is **5 to 8 minutes**.

Note: Bleeding time is lesser than clotting time, since bleeding is stopped by vascular spasm and platelet plug formation. While clotting involves a series of enzymatic reactions taking more time.

Capillary Blood Clotting Time (Wright's Capillary Glass Tube Method)

Materials and Instruments

- Materials for a sterile finger prick
- Blue banded capillary glass tubes (contain no anticoagulant,15 cm long).
- Stopwatch

Procedure:

1. Review the Safety .
2. Follow steps 1 through 9 of the “Blood Collection” section of Lab 2 to obtain a large drop of blood (Figure 8).
3. Place the sterile nonheparinized capillary tube on the drop of blood. Fill the tube at least two-thirds full with blood. Note the time when blood starts to enter the tube and start a stop watch.
4. Hold the capillary tube between the palms of your hands to keep the blood near body temperature

Procedure

5. Lay the tube on a paper towel, and gently break a small piece of the tube at the end of 2 min (Make sure you are wearing safety glasses). Repeat every 30 seconds until you notice that the blood has clotted.

Note: Clotting has occurred when a fine fibrin thread is formed between the two broken pieces of tube (figure 8).

6.Note the time. The interval between pricking the finger and the appearance of fibrin thread (clot) is the clotting time.

7.Report the results.

8.Dispose of all used materials.

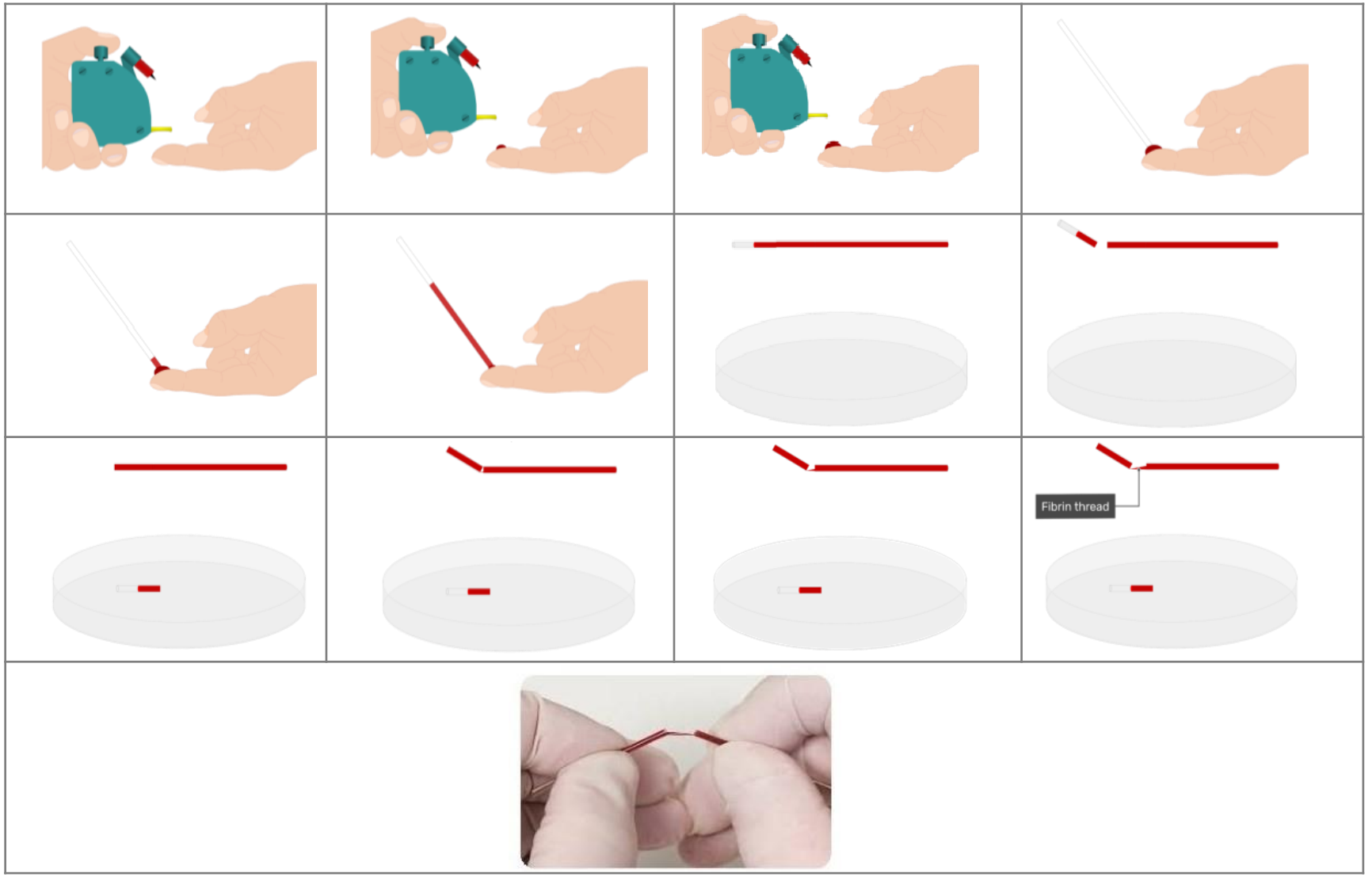


Figure 8: Determination of clotting time (Wright's capillary glass tube method).

Thank You