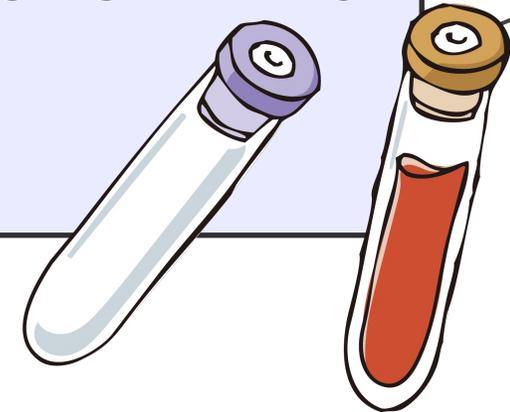
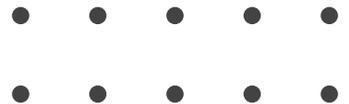
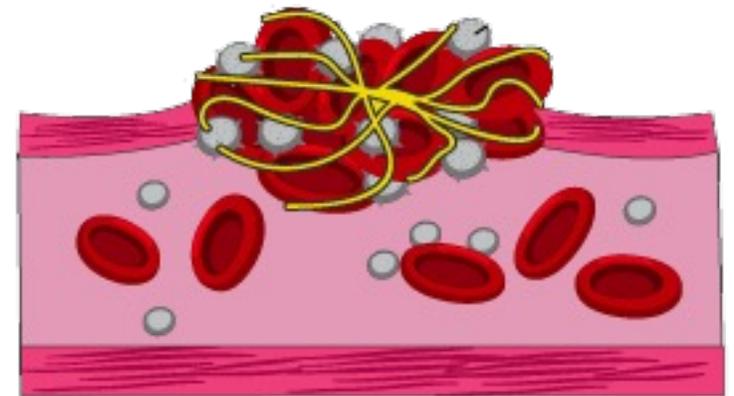
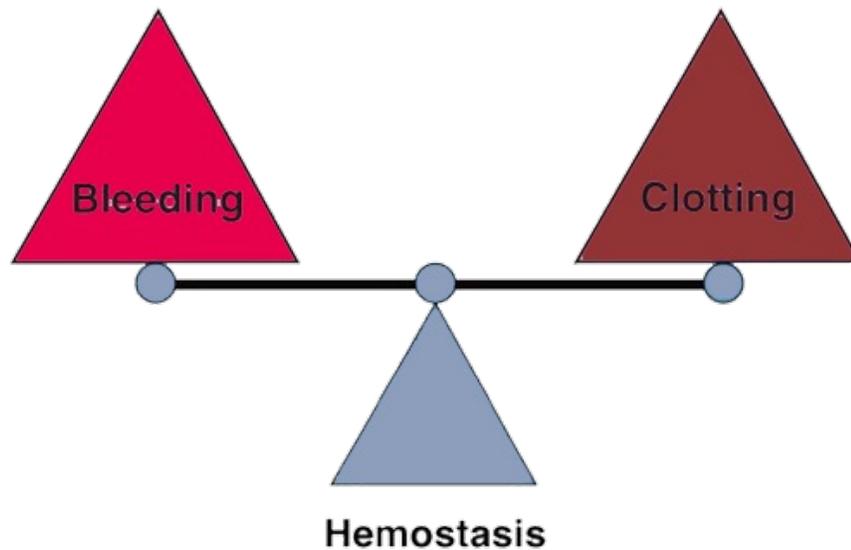


Blood Biochemistry BCH 220 [Practical]
Lab (8) Clotting Time, Bleeding Time and Prothrombin Time



Coagulation

- **Coagulation** is a complex process by which blood forms clots.
- It is an important part of **hemostasis** (the cessation of blood loss from a damaged vessel).
- Disorders of coagulation can lead to an increased risk of bleeding (**hemorrhage**) or clotting (**thrombosis**).



Hemostasis

- **Hemostasis** is a complex of homeostatic reactions, which result in arrest of bleeding from damaged blood vessels.

- Maintained in the body **via three mechanisms:**

1. Vascular spasm

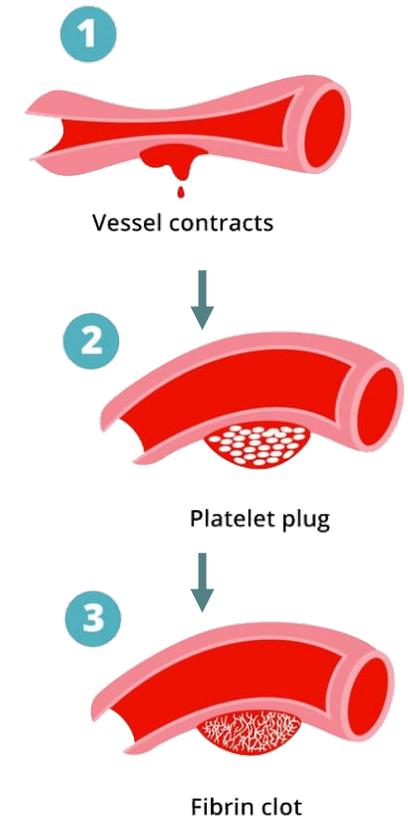
Damaged blood vessels constrict.

2. Platelet plug formation

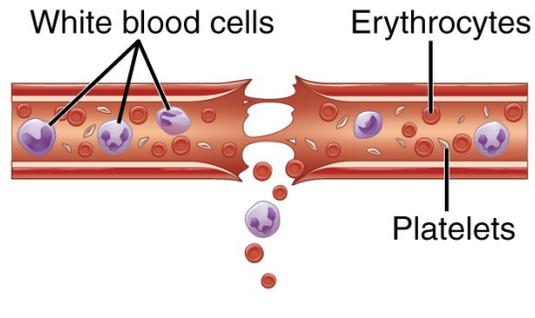
Platelets adhere to damaged endothelium to form platelet plug (**primary hemostasis**).

3. Blood Coagulation

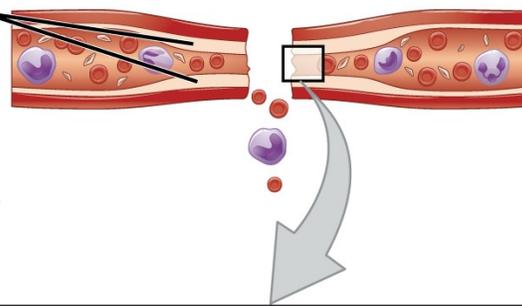
Clots form upon the conversion of fibrinogen to fibrin (**secondary hemostasis**).



① **Injury.** A blood vessel is severed. Blood and blood components (e.g., erythrocytes, white blood cells, etc.) are leaking out of the breaks.



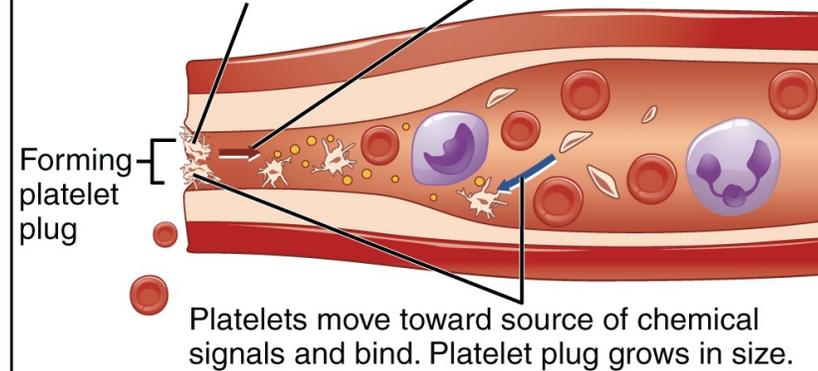
② **Vascular spasm.** The smooth muscle in the vessel wall contracts near the injury point, reducing blood loss.



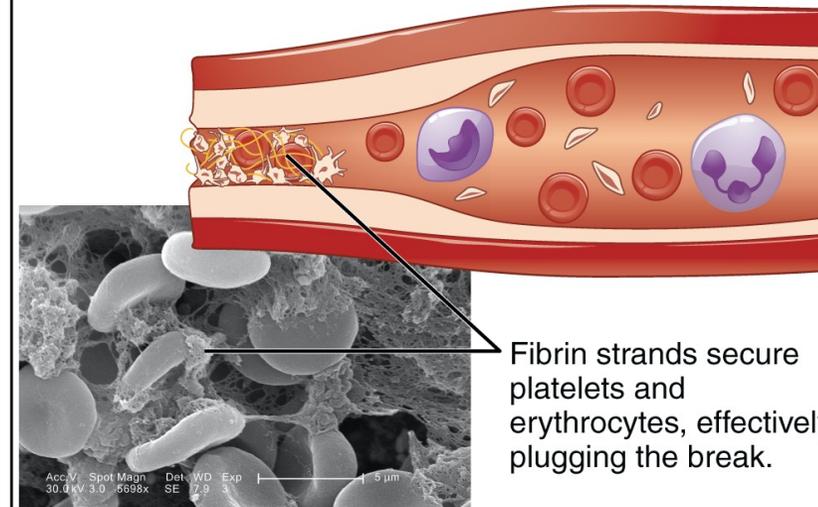
③ **Platelet plug formation.** Platelets are activated by chemicals released from the injury site and by contact with underlying collagen. The platelets become spiked and stick to each other and the wound site.

Initial platelets are activated by chemicals released from the injured cells and by contact with broken collagen.

Bound platelets release chemicals that activate and attract other platelets.



④ **Coagulation.** In coagulation, fibrinogen is converted to fibrin (see part b), which forms a mesh that traps more platelets and erythrocytes, producing a clot.



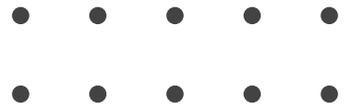
(a) The general steps of clotting

Clotting Cascade

- A **cascade** is a mechanism in which enzymes activate other enzymes sequentially usually leading to an amplification of an initial signal.

Pathways

1. **Extrinsic**, which normally is triggered by trauma.
 2. **Intrinsic**, which begins in the bloodstream and is triggered by internal damage to the wall of the vessel.
- Initially independent, then they converge on common pathway leading to the formation of a **fibrin clot**.
 - Each of these pathways leads to the conversion of factor X (**inactive**) to factor Xa (**active**).



What triggers extrinsic and intrinsic pathways?

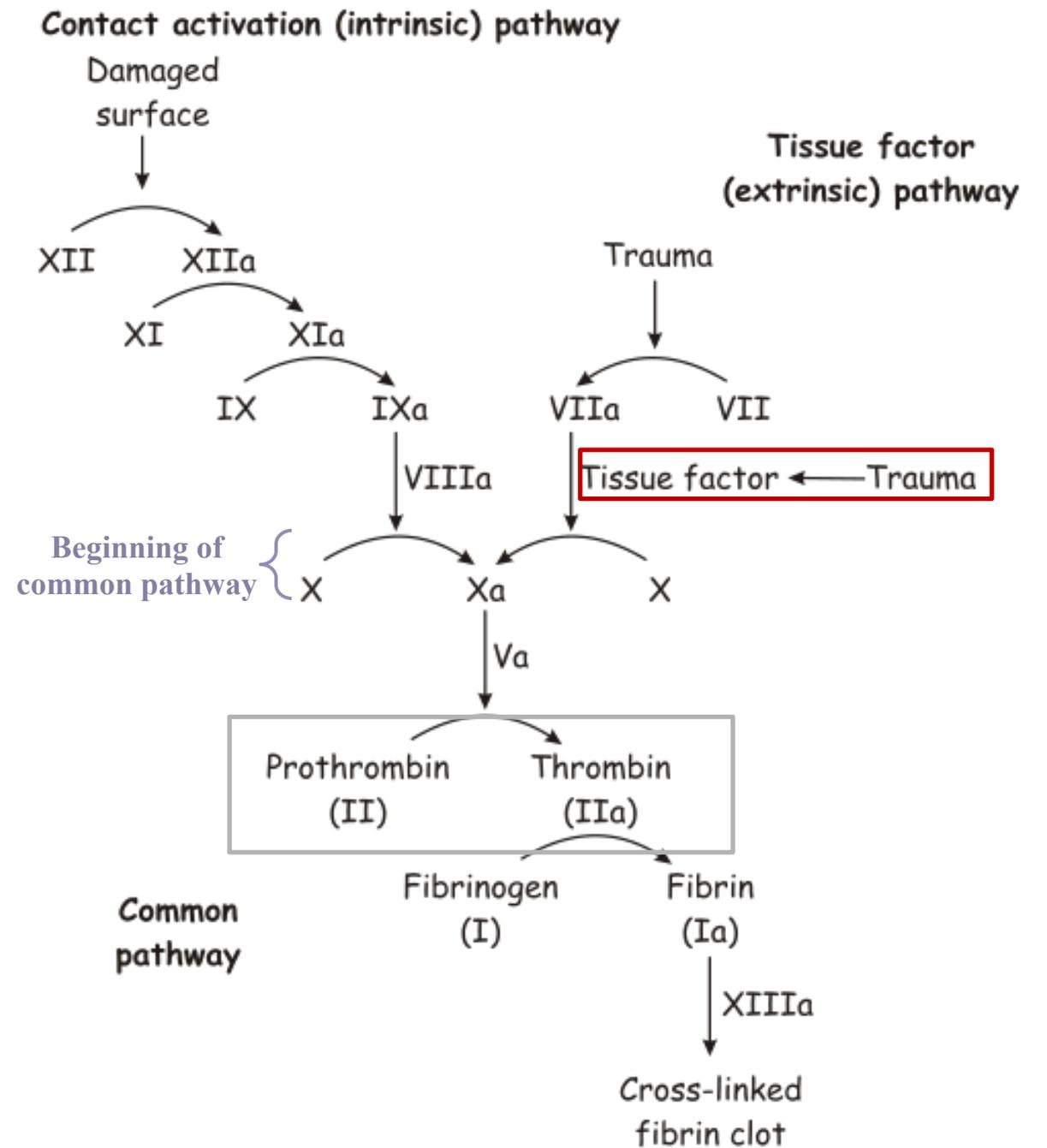
Extrinsic

- Damage to tissue **outside** the blood vessel.
- This pathway acts to clot blood that has **escaped** from the vessel.

Intrinsic

- Damage to blood vessel wall.
- Coagulates the blood **within** the damaged vessel.
- Triggered by elements that **lie within** the blood itself.

Clotting Cascade



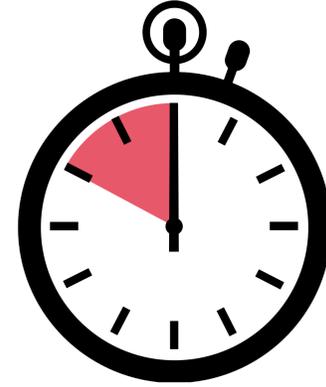
💡 **Pause and Think** why extrinsic pathway is completed in seconds whereas intrinsic pathway takes few minutes?

Practical Part



Clotting Time

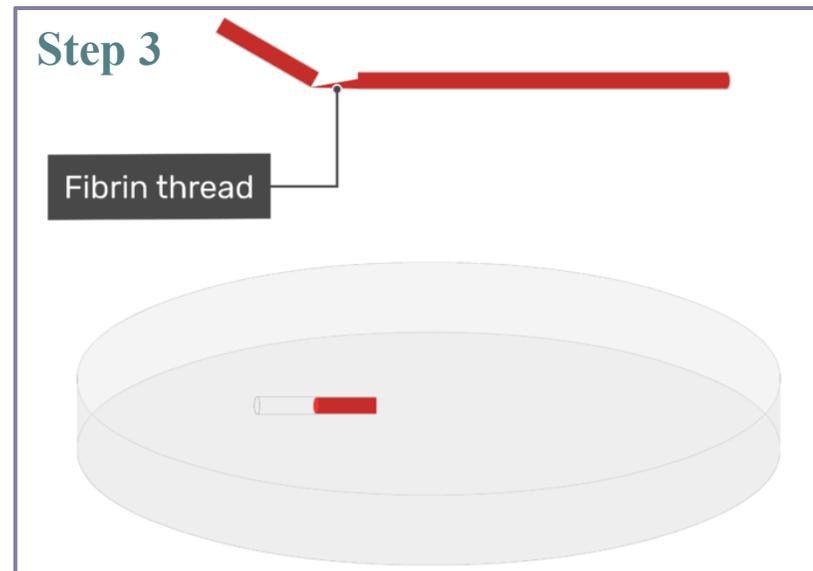
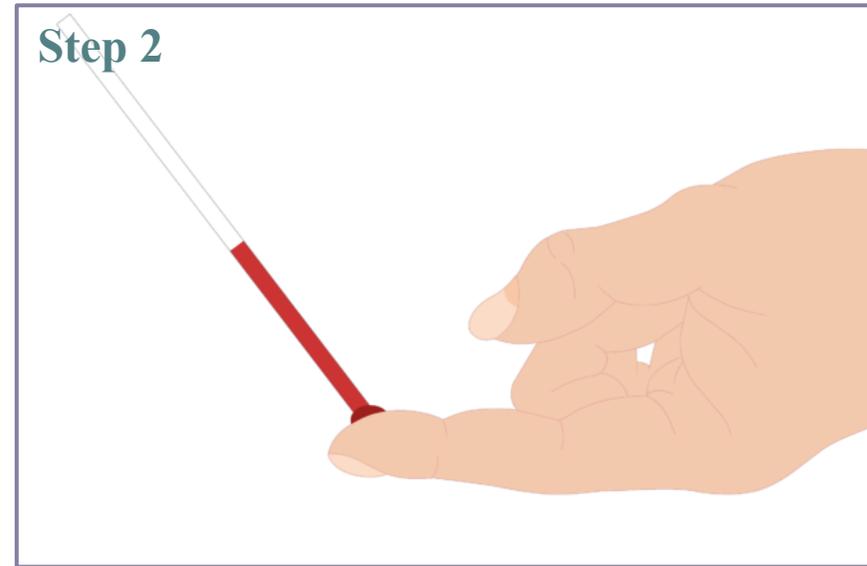
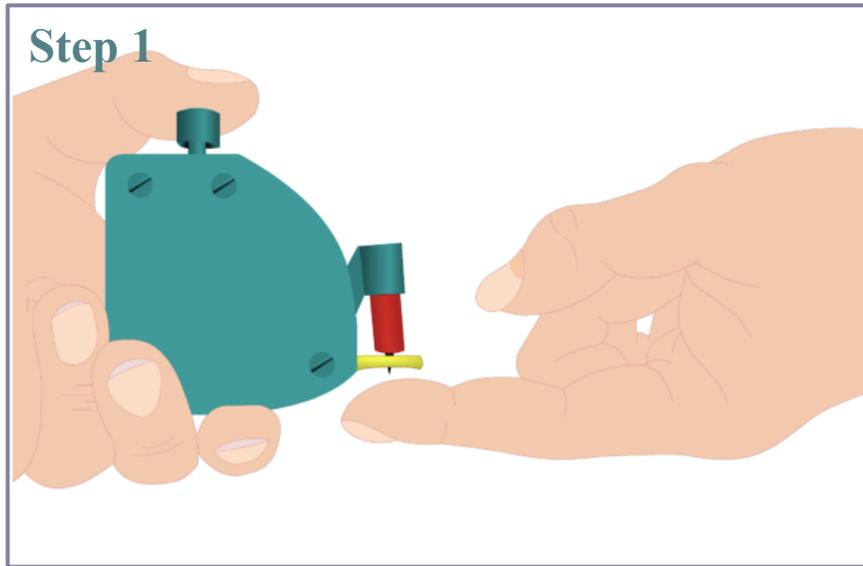
- Test for intrinsic system.
- Simple test but takes time and rarely done now.



Method:

- Venous blood is taken and placed on glass test tube at 37°C and it observed at time intervals until clotting occurs.
- Normal blood takes **5 - 10 min** to clot.
- Longer periods → Coagulation defects (e.g. Hemophilia).

Clotting Time - Capillary Method

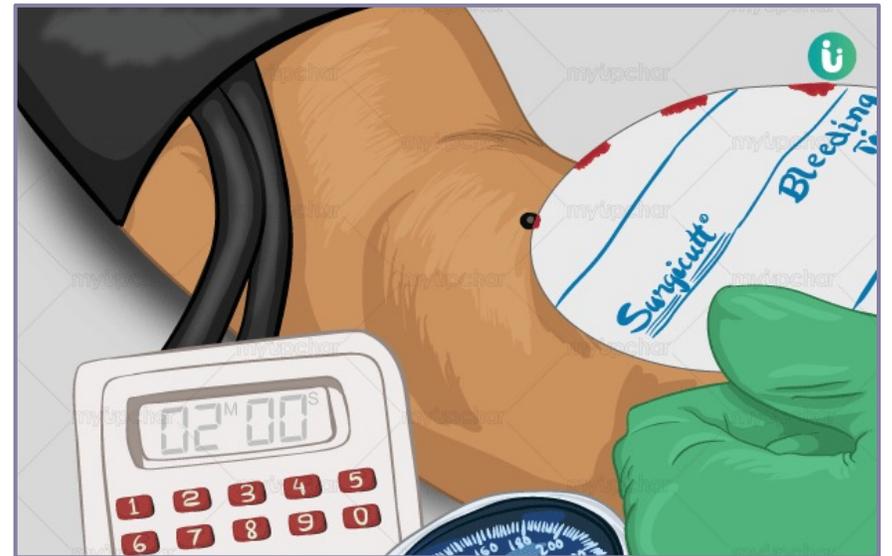


Bleeding Time (BT)

- Provides assessment of **platelet count and function.**

Method:

- It is determined by noting time at which blood coming out a small cut, no longer forms a spot on a piece of filter paper placed in contact with cut surface.
- The normal range from **2-4 min.**



Prothrombin Time (PT)

- Measures effectiveness of the extrinsic pathway

Method:

- An excess of **tissue factor** and Ca^{2+} ions are added to diluted plasma containing citrate (anticoagulant) and then the time taken for the mixture to clot is measured.
- Normal value → **10-15 secs**
- High PT** → low levels of thrombin
- Results from liver disease due to deficiency of prothrombin, fibrinogen, V, VII and X factors.

Prothrombin Time [PT]

