HEMOSTASIS

To function as a transport system, blood must be maintained in a fluid state, although it must have the ability to solidify and form a clot after a vascular injury to prevent excessive bleeding. This process is known as hemostasis. For hemostasis to be successful, clot formation should be followed by complete removal of the clot.

Hemostasis is the physiologic balance of procoagulant and anticoagulant forces that maintains both liquid blood flow and the structural integrity of the vasculature. Vascular damage results in initiation of clotting that produces a *localized* platelet-fibrin plug to prevent blood loss; this action is followed by processes that lead to clot containment, wound healing, clot dissolution, tissue regeneration, and remodeling. In healthy persons, all these reactions occur continuously and in a balanced fashion so that bleeding is contained while blood vessels simultaneously remain patent to deliver adequate organ blood flow.
If any of these processes are disrupted because of inherited defects or acquired abnormalities, disordered hemostasis may result in either bleeding or thromboembolic complications.

The process of hemostasis shows a major difference between arterial and venous injury due to the difference in rate of blood flow between an artery and a vein.
In case of arterial injury, platelets have a major role in stopping bleeding by initial plug formation which provide an active surface for soluble coagulation factors to both localize and accelerate formation of fibrin and, ultimately, clot formation.
By contrast, the slower flow rates in the venous circulation produce slower bleeding, a feature that makes platelets less critical; instead, the balance of venous hemostasis is most dependent on the rate of thrombin generation.
This concept explains the use of antiplatelets (aspirin and clopidogrel) in the prevention of coronary artery thrombosis and the use of anticoagulants (heparin and warfarin) in the prevention of venous thromboembolism.

Hemostasis is a complex process involves interaction between 3 major factors:
1- vascular endothelium
2- platelets
3- coagulation factors
A disorder of any of these factors may lead disorder in hemostasis in form of bleeding tendency or thrombosis.

1- Vascular endothelium

Vascular endothelial cells are capable of tightly controlling both procoagulant and anticoagulant events depending on circumstances.
When the vasculature is intact, the endothelial cells exert a powerful anticoagulant activity, helping to maintain blood fluidity by:

A- acting as a mechanical barrier between blood and subendothelial procoagulants.

B- secreting certain chemical substances (prostacyclin and nitric oxide) that inhibit the action of both platelets and coagulation factors.

When vascular endothelial cells are physically damaged or become activated, their balance of coagulant properties is shifted to favor a procoagulant state. They will express adhesive ligands on their surface, the main ligands are:

A- **von Willebrand factor (vWF) multimers**: localize and promote platelet adhesion.

B- **Collagen**: both a platelet ligand and a strong platelet agonist

C- **Tissue factor (TF):** the major initiator of the soluble coagulation system which, along with activated platelets, results in the formation of a definitive platelet-fibrin clot.

PROPERTIES OF ENDOTHELIAL CELL COAGULANTS

PROCOAGULANT	ANTICOAGULANT
Collagen	Vasodilation
Factor VIII	Adenosine diphosphatase
Fibronectin	Heparan sulfates
Integrins	Nitric oxide
Platelet-endothelial cell adhesion molecule-1 (PECAM-1)	Prostacyclin
Selectins (E and P)	Thrombomodulin
Vasoconstriction	Tissue factor pathway inhibitor
von Willebrand factor	Tissue plasminogen activator

2- Platelets

Platelets are anucleated cells between 2 and 4 μm in diameter, derived from the megakaryocyte cytoplasm after a maturation time of about 4 days. When platelets are released into the circulation, they survive for 7 to 10 days; platelets leave the circulation through a combination of *senescence* and the normal *maintenance* of vascular structural integrity.

The platelet functions as the cellular-based platform for hemostasis. Platelet surface receptors mediate primary hemostasis and allow platelets to bind directly to endothelium and subendothelium at sites of damage to form a plug.

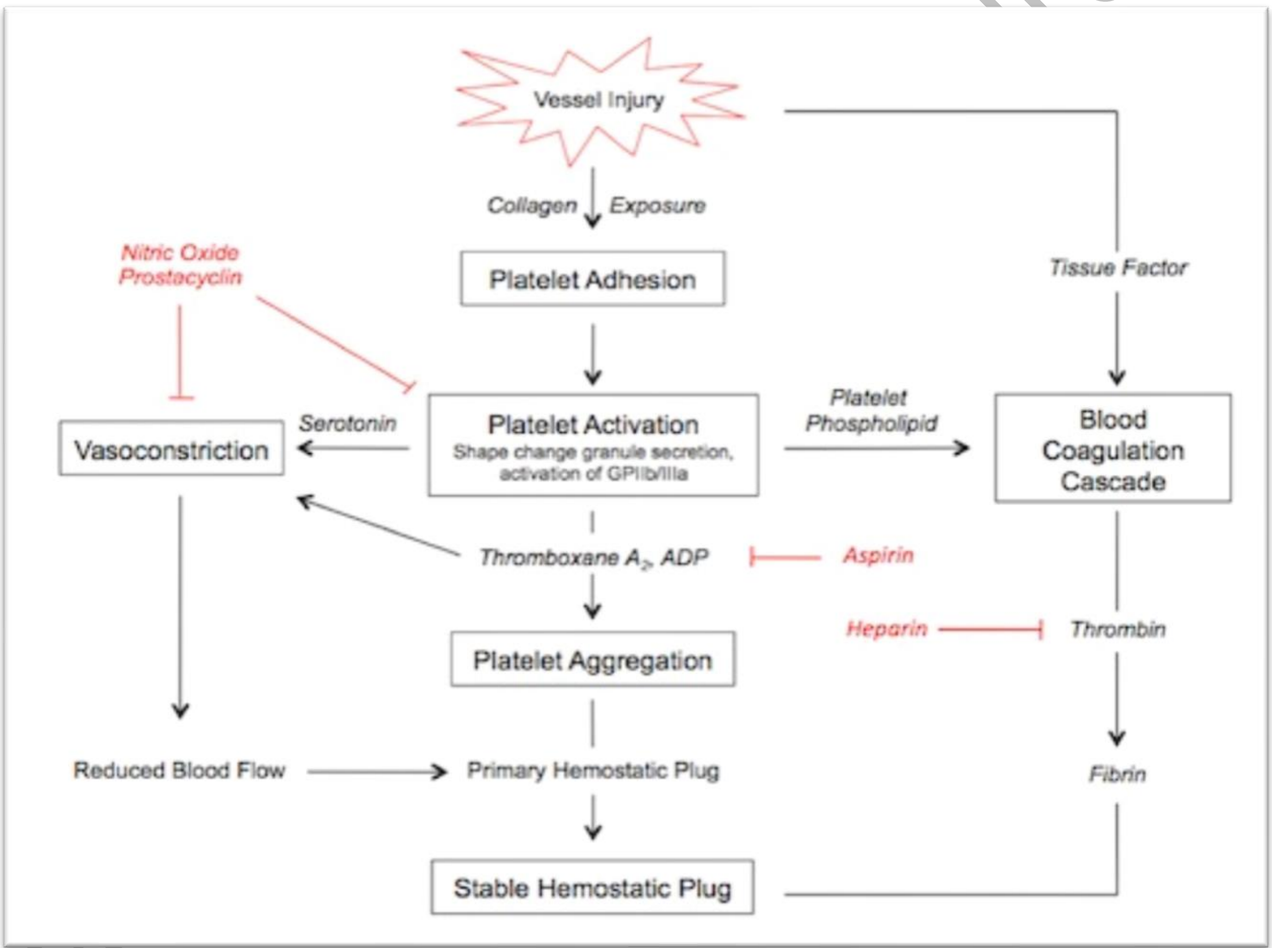
<u>The steps of primary hemostasis (platelet plug formation) are:</u>

A- platelets adhesion: occurs within seconds of endothelial injury, mediated by vWF, and acts as a bridge between glycoprotein Ib and collagen.

2- platelet activation: In this step, a platelet undergoes changes in its shape and secretes the contents of its granules. These granules contain many essential substances to complete the further steps of clot formation, examples of the contents of these granules include: (fibrinogen, fibronectin, factors V and VIII, ADP and ATP, ionized calcium, histamine, and epinephrine).

3- platelet aggregation: This step is mediated by ADP and thromboxane A_2 (TXA2) which is associated with further platelets accumulation at the site of injury and ends with hemostatic plug formation. Both ADP and TXA2 are secreted by platelet itself.

Clinical tips:
- TXA2 is both a platelet agonist and a vasoconstrictor.
- Cyclooxygenase 1 (COX1) convert arachidonic into TXA2.
- Platelet COX1 activity is irreversibly inhibited by aspirin, which blocks TXA2 formation for the lifetime of that platelet (7 DAYS).
- This is why it is better to stop aspirin 7 days before procedures expected to cause bleeding.
- Instead, NSAIDs reversibly inhibit COX1, therefore, in contrast to aspirin, the antiplatelet effects of NSAIDs are dependent on the continual presence of plasma levels of the NSAID.



3- Coagulating factors (secondary hemostasis)

Secondary hemostasis, hence coagulation factors, is essential for the formation of fibrin clot. Tissue factor of the endothelium together with secreted platelet factors will activate the coagulation cascade which represents a series of enzymatic conversions, these reactions are usually classified into intrinsic, extrinsic, and common pathway.

Two phases are present during clot formation:
First: the initiation phase, characterized by:

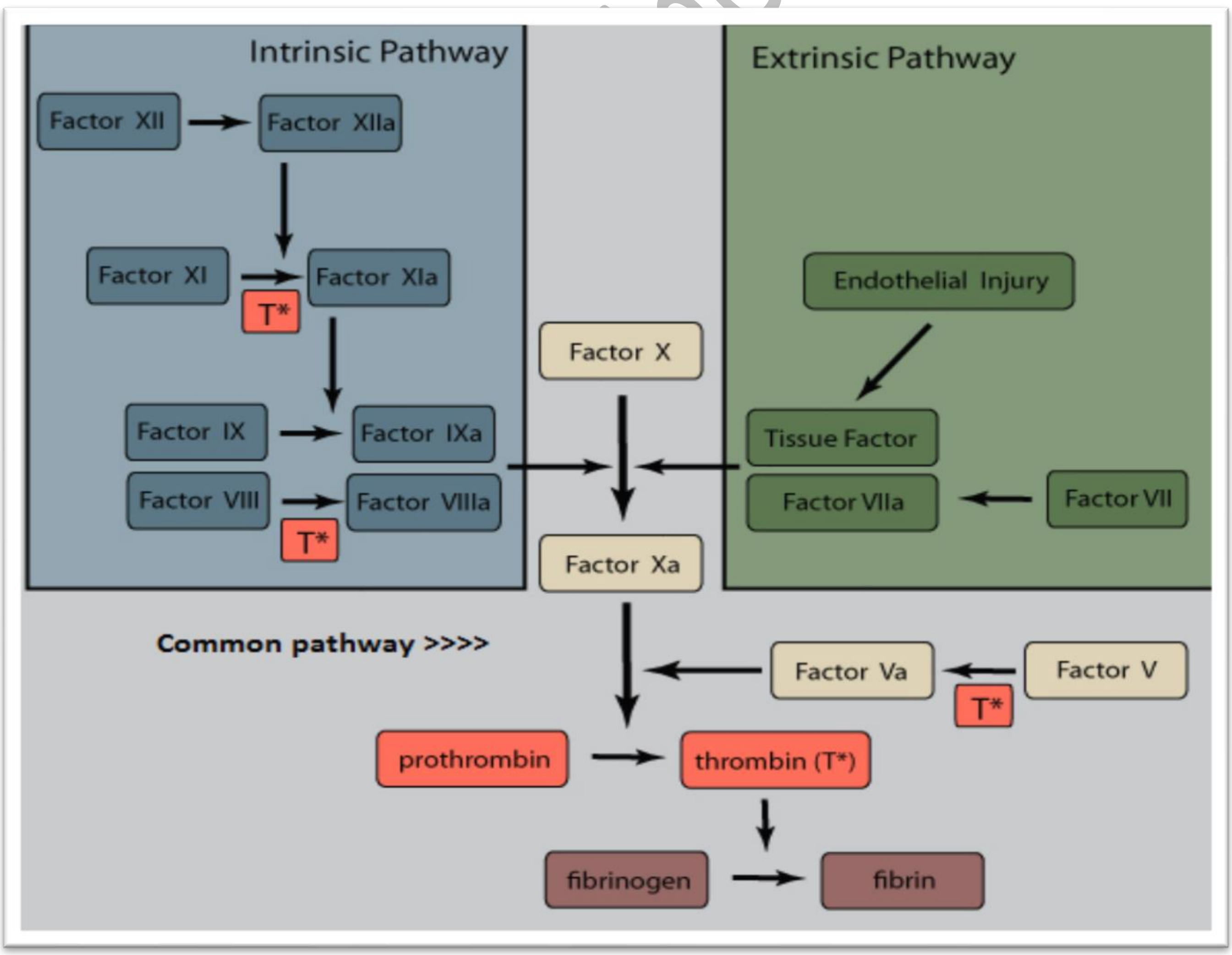
- started after exposure of blood to a source of tissue factor (endothelial injury).
- The intrinsic pathway of coagulation has no role in this phase.
- leads to the conversion of very small amounts of factor X to Xa, which in turn, generates nanomolar amounts of thrombin.
- This minimal amount of thrombin formed during the initiation phase sparks the beginning of the propagation phase, successful completion of which is characterized by explosive thrombin generation and, ultimately, fibrin deposition.
- conversion of factor X to Xa is stimulated by the TF-VIIa complex.

Second: the propagation phase, characterized by:

- More than 96% of the total thrombin that is generated during clotting occurs during the propagation phase.
- shifting of generation of Xa from the extrinsic to the intrinsic phase which is 50 times more powerful.
- conversion of factor X to Xa is stimulated by the VIIIa-IXa complex.

To summarize the differences between the intrinsic and extrinsic pathways, we can say:

Intrinsic pathway	Extrinsic pathway
Involved in propagation phase	Involved in the initial phase
Takes about 1-6 minutes	Takes about 15 seconds
Unrelated to endothelial injury	Activated by endothelial injury
Started when collagen activates factor XII	Started when tissue factor activates factor VII



Clinical tips

- Defect in secondary hemostasis usually leads to more serious bleeding than defect in primary hemostasis.

- Internal bleeding (joint, cranium, chest) is associated with secondary hemostasis defect while petechial hemorrhage is associated with primary hemostasis defect.

- Warfarin, the most widely used oral anticoagulant, is a vitamin K antagonist, it inhibits hepatic synthesis of clotting factor (II, VII, IX, X), and inhibits the extrinsic pathway.

- Following a surgical procedure, immediate bleeding indicates a defect in primary hemostasis, while delayed bleeding indicates secondary hemostasis defect.

- During a surgical procedure, hemostasis can be achieved by ways alternative to natural hemostasis, these ways include: direct pressure, ligation, or application of a hemostatic agent.

Thrombin activates the conversion of fibrinogen into fibrin which is the final step in clot formation.

Regulation of fibrin clot formation and fibrinolysis

It is essential to prevent extending of fibrin clot away from the site of endothelial injury and to prevent it from occluding healthy vessels.

So, two important mechanisms should work together to diminish any possible harmful effect of fibrin clot formation:

A- Ending the process of fibrin clot formation:

This is accomplished by the action of many substances, such as:

1- Antithrombin: which inactivate thrombin.

2- Activated protein C which inactivates factors VIIIa and Va beside many other effects.

3- Protein S: acts as a cofactor for protein C.

B- Fibrinolysis

Two factors are responsible for fibrinolysis and prevent occlusion of blood vessels by fibrin clot, these factors are secreted by endothelial cells:

1- Tissue-type plasminogen activator (t-PA).

2- Urokinase-type plasminogen activator (u-PA).

Both of these factors are capable of cleaving plasminogen into plasmin which is responsible for fibrinolysis.

Clinical tips
- Heparin, the most widely used parenteral anticoagulant, produces its major anticoagulant effect by inactivating thrombin and activated factor X (factor Xa) through an antithrombin (AT)-dependent mechanism.
- tPA are used medically in the early treatment of myocardial infarction, cerebrovascular accident, and pulmonary embolism as a fibrinolytic agent.

Laboratory Testing of Hemostasis

Because the signs and symptoms of many disorders of hemostasis are similar, performing laboratory tests is essential for diagnosis of these disorders and for monitoring patients on certain drugs.
The most important and widely used tests include:

1- Prothrombin Time (PT)

- Used to assess the extrinsic pathway.
- Highly sensitive to deficiencies of factors II, VII, V, and X (vitamin K dependent factors)
- The best test for monitoring warfarin (Coumadin) therapy.
- PT is unaffected by intrinsic pathway deficiencies of XII, XI, IX, or VIII.

2- Activated Partial Thromboplastin Time (aPTT)

- It is sensitive to deficiencies of:

  a, coagulation factors of the intrinsic pathway (XII, XI, IX, and VIII).

  b, the common pathway (V, X, and prothrombin).

- Used to monitor therapeutic heparin anticoagulation.

The PT and PTT are very sensitive for detecting congenital abnormalities associated with severe factor deficits (e.g., hemophilia) and for guiding heparin and warfarin therapy.

However, these tests fail to give information relevant to thrombin generation during the propagation phase, which determines whether a persistent clot forms or the endogenous anticoagulants and fibrinolytic regulators prevent it from excess growth.

3- Bleeding Time

- It was used to assess platelet function.
- Its clinical use is extremely diminished.

More modern techniques are present for more precise assessment of platelets function such as flow cytometry Platelet aggregometry.

4- Others:

A- vWF level assay
B- vWF activity assay
C- Coagulation factor activity assay
D- Bone marrow examination
E- Complete blood count.

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