Hemostasis

hemo= blood + stasis = stop, cease

Hemostasis is the maintenance of circulating blood in the liquid state and retention of blood in the vascular system by preventing blood loss.

THE BODY’S RESPONSE IS FAST, LOCALIZED, CONTROLLED
IT INVOLVES MANY COAGULATION FACTORS AND
THREE SEQUENTIAL STEPS

1. VASCULAR SPASM
2. PLATELET PLUG FORMATION
3. COAGULATION
VASCULAR SPASM

Immediate response is constriction of damaged blood vessels due to...

- Direct injury to vascular smooth muscle
- Chemicals released by the endothelial cells and platelets

The more tissue damage the greater the response (positive feedback system)

This reflex allows time for the platelet plug to form and clotting to occur
Intact Endothelium

When the endothelium is intact and healthy, a clot should not form. A number of factors keep a clot from forming under these circumstances.

- **Collagen**, **tissue factor** and **VWF** remain out of contact the blood due to the intact endothelium.
- **Prostacyclin** (prostaglandin I2) is synthesized by the intact endothelium and prevents platelet activation.
- **Nitric oxide** is also released under these conditions, which keeps the blood vessels dilated.
- **TFPI** (tissue factor pathway inhibitor, which is released from the endothelium, binds to tissue factor and keeps it inactive.
- **Thrombomodulin** binds thrombin and protein C, which activates protein C, which in turn inactives important clotting factors in the blood.
- **heparan proteoglycan** (heparin) binds and activates **anti-thrombin**, which in turn inactivates thrombin.
A break in the endothelium allows platelets to contact collagen and the other factors that activate platelets. Platelets begin adhering to one another and the subendothelial tissue via fibrinogen and VWF receptors. If the break is small, this platelet plug may be adequate to seal the break.
Exposed collagen binds and activates platelets.
Platelet factors are released.
Factors attract more platelets.
Platelets aggregate into platelet plug.
B. PRIMARY HEMOSTASIS

1. Platelet adhesion
2. Shape change
3. Granule release (ADP, TXA₂)
4. Recruitment
5. Aggregation (hemostatic plug)

Key:
- Endothelium
- Basement membrane
- Collagen
- vWF
A good model should also be complicated enough to reflect the realities of the biological system.

"I think you should be more explicit here in step two."

With apologies to Sidney Harris.
Platelets remain disc shaped and inert in a normal environment, when epithelium is disrupted.
Exposing collagen, platelets become ACTIVATED the following sequence occurs...

- ADHESION
- ACTIVATION
- PRIMARY AGGREGATION
- SECRETION
- SECONDARY AGGREGATION

Factors that will cause platelet activation are:
- Exposure to collagen from vascular injury
- ADP (causes the platelet membrane to shift and expose receptor sites)
- Platelet activation factor (released by monocytes and macrophages)
- Thrombin (is activated prothrombin)
- Epinephrine (platelet aggregation)
- Thromboxane A (prostaglandin=platelet aggregation)
NAVIGATING THE COAGULATION CASCADE
COAGULATION IS A MULTISTEP PROCESS

GOAL:
IS TO SEAL THE BREAK IN THE BLOOD VESSEL WALL WITH A FIBRIN MESH
TO ALLOW PERMANENT REPAIR OF VESSEL WALL TO OCCUR

OVER 30 DIFFERENT SUBSTANCES INVOLVED:
FACTORES THAT ENHANCE CLOTTING FORMATION ARE CALLED
PROCOAGULANTS FACTORS THAT INHIBIT CLOTTING ARE CALLED
ANTICOAGULANTS

NOTE: THERE IS MORE ANTICOAGULANTS THAN COAGULANTS AND
CLOTTING IS PREVENTED. WHEN VESSELS RUPTURE PROCOAGULANT ACTIVITY INCREASES
DRAMATICALLY TO PREVENT BLOOD LOSS.
Coagulation Reactions Begin at Surface of Platelet

Coagulation reactions begin occurring more rapidly since tissue factor is exposed and the surface of activated platelets provides the environment for the activation of the cascade that ultimately converts prothrombin to thrombin. The developing clot consists of interlaced fibrin fibrils and activated platelets.
Receptor sites for:
Fibrinogen, fibronectin, vonWillebrand F, epinephrine, thrombin, ADP, serotonin.
Platelet factors PF1-PF7 & coagulation FV and VIII are found on membrane.

Means for releasing stored products:
Calcium storage and release.

Form pseudopods:
Cause platelet contraction.

Calcium storage and release:

Contains Glycoprotein lb, Receptor for vWF & Glycoprotein IIa/IIa
Receptor for fibrinogen.

Peripheral Zone (Adhesion interface)
- Glycocalyx (exterior coat)
- Phospholipid Membrane (neg. for F10 and prothrombin activator)
- PF-3 moves membrane to exophospholipids to Vit-K cofactor
- Various other platelet factors

S-C-S = Open Cannicular System

Sol-Gel Zone (Cytoskeleton)
- Microtubules of actin and myosin interwoven throughout cell
- Contraction = shape change

Organelle Zone (metabolic activities)
- No nucleus
- Dense Granules: ADP, ATP, Ca++, serotonin, phospholipids

Platelet Morphology
Platelet Activation

When a platelet encounters a break in the endothelium, it encounters molecules that trigger its activation. One such molecule is collagen, which is characteristically found almost everywhere except inside a blood vessel. In addition, thromboxane A2, ADP and thrombin are other factors that trigger the same activation.

activation of the membrane enzyme phospholipase A2, leading to the formation of thromboxane A2 (TXA2)
SHAPE CHANGE

Increases cytoplasm calcium (Ca++)

1) Microtubules in cytoplasm contract
2) Pseudopods form
3) Organelles move to center of platelet
4) Fibrinogen receptor changes shape and can now bind to fibrinogen

Collagen

ADP

Platelet

Thrombin

Thrombin starts platelet activation; causes release of ADP and TXA2

Platelet

TXA2 (prostanadion)

GPIIb/IIIa receptor (Inactive fibrinogen receptor)

Thrombin promotes secretion and aggregation

Secretion and aggregation

Fibrinogen receptor changes shape and can now bind to fibrinogen

Open Cunntular System (OCS)

α granules

Dense granules

GPIIb/IIIa

Fibrinogen binds platelets together
Aggregation

(Platelet to Platelet Interaction)

10-20 seconds after injury

Requires
1. ATP from glycolysis
2. Ca^{++}
3. Fibrinogen receptors
4. Fibrinogen

ADP induces exposure of fibrinogen receptors

Thrombin initiated platelet activation

Fibrinogen (from plasma)
TWO PATHWAYS TO PROTHROMBIN ACTIVATOR

PIVITOL COMPONENT IN BOTH PATHWAYS IS NEGATIVELY CHARGED MEMBRANES ESPECIALLY THOSE ON THE PLATELETS CONTAINING PLATELET FACTOR PF3

SLOWER PATHWAY ALL FACTORS ARE PRESENT IN THE BLOOD

FASTER PATHWAY WHEN EXPOSED TO TISSUE FACTOR

Intrinsic Pathway

Surface Contact Collagen FXII activator

F XII → F XIIa

F XI → F XIa

F IX → F IXa

Factor F X → Ca²⁺

Extrinsic Pathway

Tissue/Cell Defect

F VIIa → Ca²⁺ → F VII

F III (Tissue Thromboplastin) → Ca²⁺ → Factor F X
The Clotting Cascade

Intrinsic Pathway

Surface Contact Collagen FXII activator

F XII → F XIIa

F XI → F Xla → Ca^{2+} → F IXa → F IX

F VIII → F VIIIa → Platelet Factor 3 → F IXa → Ca^{2+} → F X

Factor F X

Extrinsic Pathway

Tissue/Cell Defect

F VII → F VIIa → Ca^{2+} 

F III (Tissue Thromboplastin) → Factor F X → Ca^{2+} 

F VIIa → F Xla

Factor F Xa

Prothrombin I → Ca^{2+} → Thrombin

F Xla → F XIIIa

F XIII → Crosslinked Fibrin Meshwork

Fibrin monomers → Fibrin polymers → Fibrinogen
Spasm in damaged smooth muscle

1. VASCULAR PHASE

Platelet aggregation and adhesion

2. PLATELET PHASE

**COMMON PATHWAY**

**INTRINSIC PATHWAY**
- Platelet thromboplastin
- Prothrombin → Thrombin
- Fibrinogen
- Fibrin
- Clotting factors VIII, IX, X, XI, XII
- Platelet factors
- Ca²⁺

**EXTRINSIC PATHWAY**
- Tissue thromboplastin
- Tissue factors
- Ca²⁺
- Clotting factor VII

**3. COAGULATION PHASE**
- Activation of clotting system and clot formation

**Actin & myosin**

**Contract**
- PDGF

4. CLOT RETRACTION
- Contraction of blood clot

**5. CLOT DESTRUCTION**
- Enzymatic destruction of clot

**Plasminogen**
The Fibrinolytic System

 Begins within two days cont. slowly over several days

Clotting Cascade

Fibrinogen → Fibrin-clot → Fibrin degradation products

Plasminogen → Plasmin

(Fibrin digesting enzyme when plasminogen activated)

Activated FXII and thrombin release
During clotting

(Lrg. amounts of plasminogen
Incorporated into a forming clot remains
Inactive until signaled)

(Tissue plasminogen activator produced
By endothelial cells)

T-PA
F Xlla
HMWK
Kallikrein
Urokinase
Streptokinase
FIBRINOLYSIS

Plasminogen Activator Inhibitor

Thrombin
Mediates Anticoagulation

Plasminogen (in plasma)

Plasmin (enzyme)

t-PA

Breaks down Fibrin

Res System (Liver)

Macrophages Phagocytize

Fibrin and Activated Factors

Thrombin-promotes healing

Alpha granules:
- β-TG = vessel repair
- PDGF = smooth muscle repair
FACTORS LIMITING CLOT FORMATION

HEOMEOSTATIC MECHANISMS THAT PREVENT CLOTS FROM BECOMING TOO LARGE

• SWIFT REMOVAL OF CLOTTING FACTORS BY RAPIDLY MOVING BLOOD
• AS A CLOT FORMS ALL THROMBIN IS BOUND BY FIBRIN
• INACTIVATING THROMBIN BY ANTITHROMBIN III & PROTIEN C
• HEPARIN NATURAL ANTICOAGULANT IN BSOPHILS AND MAST CELL GRANULE
• HEPARIN IS ALSO PRODUCED BY ENDOTHELIAL CELL
Coagulation proteins

**Prothrombin Group**
- Vit. K dependant produced in the liver
- All are Serine proteases
  - Prothrombin FII
  - Proconvertin FVII
  - Christmas Factor FIX
  - Stuart-Prower Factor FX

**Fibrinogen Group**
- NOT Vit. K dependant, produced in the liver
  - Fibrinogen FI (substrate)
  - Proaccelerin FV (co factor)
  - Antihemophilic Factor FVIII (co factor)
  - Fibrinogen stabilizing FXIII (serine protease)

**Contact type coagulation Factors**
- Involved in the initial activation of the intrinsic coagulation
  - Plasma thromboplastin antecedent (XI)
  - Hageman Factor (XII)
  - Pre-Kallikrein (PK)
  - High -molecular-weight kininogen (HMWK)
<table>
<thead>
<tr>
<th>Factor</th>
<th>Common Name (with known synonyms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>II</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>III</td>
<td>Tissue Thromboplastin; Tissue Factor</td>
</tr>
<tr>
<td>IV</td>
<td>Ionized Calcium (Ca^{++})</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin, Labile Factor, Prothrombin Accelerator, Accelerator Globulin (AcG), Thrombogen</td>
</tr>
<tr>
<td>VI</td>
<td>This designation is now unassigned. It was formerly listed as a clotting factor, it is now known that it was an impure derivative of Factor V. It is now a discarded term and textbooks do not list this factor nor indicate it as being unassigned.</td>
</tr>
<tr>
<td>VII</td>
<td>Stable Factor, Serum Prothrombin Conversion Accelerator (SPCA), Proconvertin</td>
</tr>
<tr>
<td>VIII</td>
<td>Antihemophilic Factor (AHF), Antihemophilic Factor A, Antihemophilic Globulin (AHG), Platelet Cofactor 1.</td>
</tr>
<tr>
<td>X</td>
<td>Stuart-Prower Factor, Stuart Factor, Autoprothrombin III, thrombokinase.</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma Thromboplastin Antecedent (PTA),, Antihemophilic Factor C, Prothrombin Antecedent</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman Factor, Contact Factor, Glass Factor</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin-Stabilizing Factor (FSF), Transglutaminase, Fibrinoligase, Laki-Lorand Factor (LLF), Fibrinase, Plasma Transglutaminase</td>
</tr>
<tr>
<td></td>
<td>Fitzgerald High-Molecular-Weight Kininogen (HMWK), Flaujeac Factor, Contact Activation Factor, Williams Factor, Reid Factor, Washington Factor</td>
</tr>
<tr>
<td></td>
<td>Fletcher Prekallikrein (PK)</td>
</tr>
</tbody>
</table>
QUESTIONS AND ANSWERS

1. WHAT DO ENDOTHELIAL CELLS PRODUCE TO KEEP PLATELETS FROM BEING ACTIVATED?

   PROSTACYLIN, NITRIC OXIDE,
   **TFPI** (tissue factor pathway inhibitor)

2. WHAT FACTORS WILL ACTIVATE PLATELETS
   - Collagen, **tissue factor** and **VWF** in contact with the blood due to vessel wall

3. What are the three steps that occur in rapid sequence when there is damage to Vessel wall?
   1. vascular spasm,
   2. platelet plug formation
   3. coagulation of blood

4. What are the two pathways to the common pathway
   - Intrinsic with the involvement of collagen, PF3, factors: XII, XI, IX, VII with VWF (calcium)
   - Extrinsic with the involvement of TF3, FVII (calcium)
   - Both leading to the common pathway has factor PF3, V, X, II, I, XIII (calcium)