Cell based model of haemostasis

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Summary: The complex process of coagulation that leads to hemostasis has been described by several models in the past, the most popular of which was the cascade model. Though the cascade model (with intrinsic, extrinsic and common pathway) was a good description of this process in vivo, it fails to account for several experimental and clinical findings that have come to light in the last few decades. The in vitro activation of the coagulation process (the basis of the cascade model) doesn’t completely represent the global haemostatic process that occurs in vivo, characterised by interactions between tissue factor bearing cells, platelets and the intrinsic and extrinsic pathways. The cell-based model of haemostasis is the currently accepted model as it better reflects the process of haemostasis in vivo. It consists of three overlapping phases – initiation, amplification and propagation phases. It overcomes many of the limitations of the cascade model and is clinically relevant.

Introduction

Haemostasis is the process by which bleeding is stopped after an injury by the formation of a clot, while at the same time, maintaining blood in a fluid state elsewhere. Injury to a blood vessel results in vasoconstriction and temporary platelet plug formation. This is followed by a coagulation process which arrests bleeding at the site of injury by forming a fibrin clot. The clot formed at the site of injury by the coagulation process can potentially spread to the adjacent normal areas leading to clotting of blood elsewhere. This is prevented by the presence of fibrinolytic system and various natural anticoagulants in the intact blood vessel.

Our understanding of the process of haemostasis has evolved over years through various models. The cascade/waterfall model of haemostasis (which includes an intrinsic pathway, extrinsic pathway and a common pathway) has been in use for several decades and is the most popular. Although useful in our understanding of haemostasis, this model does not fully explain several clinical and experimental findings. The cell-based model of haemostasis (initially proposed by Hoffman, Monroe et.al. and later expanded by K.G. Mann, S. Butenas et al.), which proposes that clotting occurs not as a cascade, but in three overlapping stages is the currently accepted model of haemostasis that adequately reflects the process of clotting. The current article will update the readers about the cell based model of haemostasis.

Models of haemostasis

The early understanding of the process of clotting during the times of Hippocrates, Aristotle, Celsius and Galen was that bleeding stopped when blood came into contact with air. They hypothesised that blood cooled on contact with air resulting in the cessation of bleeding. The formation of blood clots within vessels was described in the early 1720s by the French surgeon Jean-Louis Petit and in 1860, the German pathologist Rudolf Virchow described thrombi and their tendency to embolise.

It was only in 1905 however that a model to describe the process of haemostasis was proposed by Paul Morawitz. He described the four factor model of haemostasis where prothrombin was converted to thrombin by calcium. The thrombin formed then converted fibrinogen to fibrin (Figure 1). This model was also known as the classic theory of haemostasis. However this model

![Figure 1: Classic theory of haemostasis](image-url)
was not able to explain bleeding tendencies in patients who had normal levels of these factors. Later, other clotting factors were discovered in the blood. These clotting factors were assigned Roman numerals based on the order of discovery. Based on these discoveries and identification of bleeding diseases, two independent groups proposed a cascade and waterfall model of haemostasis in 1964 (4,5). These models formed the basis for the intrinsic and extrinsic pathways described in most medical textbooks.

**The cascade model of haemostasis**

The cascade/waterfall model suggests that clotting factors are in an inactive proenzyme form and they get activated sequentially by a series of proteolytic reactions (in the intrinsic and extrinsic pathways) during the coagulation process to finally generate a burst of thrombin1. These two pathways eventually resulted in the activation of the common pathway (Figure 2).

**Intrinsic pathway**

The intrinsic pathway was named so because all the factors required for this pathway were present in blood. The intrinsic pathway is activated when factor XII comes in contact with negatively charged subendothelial collagen which is exposed following injury to the blood vessel and also by cell and platelet derived polyphosphates6. During this process factor XII is converted to its active from factor XIIa. This is followed by a sequential cascade of activation of Factor XI, IX and X to their active forms XIa, IXa and Xa. Factor Xa along with factor Va, calcium and platelet phospholipids form prothrombinase2 which then results in the formation of fibrin through the common pathway (figure 2).

The intrinsic pathway is tested in the laboratory by measuring activated partial thromboplastin time (aPTT).8

**Extrinsic pathway**

The extrinsic pathway requires tissue factor present in subendothelial cell membranes. This pathway is activated when factor VII in blood comes in contact with tissue factor (TF) that is released following tissue injury.2 Factor VII and factor X are converted into their active forms VIIa and Xa. Factor Xa along with factor Va, calcium and platelet phospholipids form prothrombinase which then results in the formation of fibrin through the common pathway (figure 2).

The extrinsic pathway is tested in the laboratory by measuring the prothrombin time (PT).5

**Common pathway**

The common pathway is similar to the four factor model of haemostasis where prothrombin or factor II is converted to thrombin (IIa) by prothrombinase3. The prothrombinase consists of activated factor X (Xa), activated factor V (Va), calcium and platelet phospholipids. The thrombin that is formed then initiates the formation of a stable fibrin plug.

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Fig. 2 – The cascade model of haemostasis

Ca²⁺: Calcium ion, HMWK – High molecular weight kininogen.

TF – Tissue factor
The factors in common pathway (factors X, V, II and I) can be tested in the laboratory by measuring the aPTT and PT.\(^8\)

**Limitations of cascade/waterfall model**

The cascade/waterfall model is good for describing the coagulation process *in vitro* (in the laboratory) where we can selectively activate the intrinsic or extrinsic pathways\(^1\). However several clinical observations have exposed its limitations while describing the coagulation process *in vivo*. Some of the observations are:

1. It has been observed that individuals with factor XII deficiency do not suffer from bleeding in spite of the requirement of this factor for initiating the intrinsic pathway \(^9\). The aPTT of these individuals are prolonged but they are asymptomatic.
2. Deficiency of high molecular weight kininogen and pre-kallikrein also do not lead to a clinical bleeding tendency\(^1\).
3. Deficiency of factor XI leads to variable haemostatic deficits in human beings with bleeding seen in some individuals \(^8\).
4. Individuals with factor IX or factor VIII deficiency have severe bleeding even though their extrinsic and common pathways are normal and should be sufficient to promote clotting.\(^7\)
5. Deficiency of factor VII also causes bleeding even though the intrinsic pathway is intact. This means that intrinsic and extrinsic pathways alone cannot bring about haemostasis and an interaction between both pathways is necessary for haemostasis *in vivo*. This interaction can be explained by the cell based model of haemostasis.

**The Cell based model of haemostasis**

The cell based model of haemostasis gives a better description of the process of haemostasis as it occurs in the body and better reflects this process when compared to the cascade model.

The formation of thrombin from prothrombin is the central event in the process of coagulation which results in clotting of blood. There are several reactions which precede thrombin production. These reactions occur rapidly and do not follow a neat sequence of events as described by the cascade model. For the purpose of understanding these events, the process of coagulation may be divided into three phases which are interwoven and overlap each other.

The phases of coagulation are:

1. Initiation phase
2. Amplification phase
3. Propagation phase

**I. Initiation phase**

Tissue factor (TF) is the initiator of the process of haemostasis. TF is normally present in TF-bearing cells like smooth muscle cells and fibroblasts in the subendothelial layer of blood vessels and a small amount in macrophages, endothelial cells and platelets circulating in the blood\(^2,6\). It is hidden and membrane bound and is expressed on the surface of these cells only after an injury.

The processes involved in the initiation phase, which takes place on the surface of these TF bearing cells, are as follows: \(^1,2,8\)

i. **Binding of TF with factor VII**

The coagulation process is initiated by the contact of factor VII (or activated factor VIIa) present in the plasma with tissue factor (TF) exposed on the surface of TF bearing cells.\(^1\) This contact occurs when the endothelium is breached by the injury.

ii. **Activation of Factor X and IX**
The factor VIIa/TF complex then activates factor X to factor Xa and factor IX to factor IXa providing interaction between the extrinsic and intrinsic pathways.

iii. Conversion of Prothrombin to Thrombin

Factor Xa activates the conversion of prothrombin to thrombin (figure 3).

Only a small amount of thrombin is formed in this phase because of some inhibiting factors - the circulating Xa is inhibited by the binding of endothelium derived tissue factor pathway inhibitor (TFPI) and by antithrombin III (ATIII) present on normal endothelium. The TFPI/Xa complex also inhibits the VIIa/TF complex.

The formation of thrombin during this phase is very inefficient and very little is formed. However this small amount of thrombin is necessary to initiate the amplification phase.

II. Amplification phase

The amplification phase occurs on the surface of platelets. The processes involved are as follows.

i. Activation of platelets

Platelets now adhere to the injured vasculature and extra-vascular tissues (mediated by von Willebrand factor). The small amount of thrombin formed from the initiation phase on the TF bearing cells acts on these platelets converting them into activated platelets.

Components of platelet activation:

a) Platelets become irregular in shape with multiple pseudopodia that increase surface area for the coagulation process.

b) Platelets start expressing receptors and binding sites for various clotting factors. This is done by reversing the asymmetry of the phospholipid bi-layer of the platelet membrane. The anionic phosphatidyl serine (PS) found on the inner layer is brought to the outer layer by a process referred to as “flip-flop”. The anionic PS attracts the positively charged Ca$^{2+}$ ions involved in the formation of tenase complex (described later).

c) Platelets start releasing serotonin, calcium and ADP from their dense granules. They also release factor V, fibrinogen, von Willebrand factor (vWF) and platelet derived growth factor from their alpha granules.

ii. Activation of Factors V, VIII and XI

a) The factor V on the platelet surface gets activated by thrombin produced during the initiation phase.

b) Thrombin also activates factor VIII present on the platelet surface complexed with vWF. During this process, vWF/VIIIa complex separates and the vWF then mediates further platelet adhesion and aggregation. Activated factor VIIIa will remain on the platelet surface.

c) Thrombin also activates Factor XI present on platelet surface (figure 4).

The activated platelets and factors Va, VIIIa and Xla on the platelet surface thus set the stage for the next stage of the amplification phase which is characterized by the formation of two coagulation complexes - tenase and prothrombinase.

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Figure 4: Amplification phase.

vWF: von Willebrand factor
iii. Formation of \textit{tenase} complex

The activated factor IXa combines with factor VIIIa (along with calcium and phospholipids) on the platelet surface to form the \textit{tenase complex} (VIIa/IXa) which converts the inactive factor X (substrate) into activated factor Xa.\(^2\)

iv. Formation of \textit{prothrombinase} complex

Activated factor Xa now forms a complex with factor Va (along with calcium and phospholipids) on platelet surface to form \textit{prothrombinase complex} (figure 5).

v. Formation of thrombin

The \textit{prothrombinase} (Xa/Va) converts prothrombin (substrate) into large amounts of thrombin\(^{13}\). When compared to factor Xa (in the initiation phase), prothrombinase is about 300,000 times more active in the formation of thrombin from prothrombin.\(^{14}\) This large scale formation of thrombin is often referred to as ‘burst of thrombin’.

III. Propagation phase

Further production of thrombin:

Factor Xla that was formed in the initial part of the amplification phase (inactive factor XI activated by thrombin) in turn activates factor IX to factor IXa which enables the formation of \textit{tenase} complex leading to the formation of thrombin (The amount of thrombin formed during the amplification phase could be sufficient for clot formation, in which case this phase of thrombin formation may not be critical.)

Formation of thrombin and fibrin plug:

The \textit{prothrombinase} initiates a burst of thrombin formation from prothrombin. The thrombin converts fibrinogen to fibrin monomers. These fibrin monomers, in the presence of factor XIIIa, interweave with each other and platelets to form a stable fibrin plug that seals the wound and stops bleeding.

Control and Termination of clotting

If the process of thrombin production is not controlled, the thrombin that is formed at the site of injury can spread to the adjacent normal areas and cause coagulation in surrounding normal areas. The process of thrombin formation is therefore under tight control by various mechanisms mediated by factors like TFPI (through processes described earlier) and natural anticoagulants like Protein C, Protein S and Antithrombin III which activate the fibrinolytic system. The activated antithrombin III complex can inactivate thrombin and other clotting factors like IX, X, XI and XII.

Drugs like streptokinase, tissue plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA) act by activating the fibrinolytic pathway and are useful in lysing clots in patients with myocardial infarction, ischaemic stroke, deep vein thrombosis and pulmonary embolism.

Clinical implications

1. Normal haemostasis requires normal vascular function, normal platelets and coagulation factors. Blood vessel abnormalities and a decrease in platelet count or platelet dysfunction can lead to bleeding tendencies. Coagulation factor deficiencies
can also lead to bleeding disorders like haemophilias.

2. **Deficiency of factor VII** can lead to bleeding as it is important in the initiation phase of haemostasis.

3. **Haemophilia A or classic haemophilia** is due to deficiency of clotting factor VIII. Deficiency of clotting factor IX results in Haemophilia B or Christmas disease. Both haemophilia A and B are inherited as X linked recessive disorders. Usually males are affected and females are carriers. Haemophilia A and B are common in communities that promote consanguineous marriage. Factor VIII and IX deficiencies lead to severe bleeding as they are important for the amplification phase of haemostasis.

4. **Haemophilia C** is due to deficiency of factor XI and is inherited as an autosomal disease. Factor XI deficiency causes variable bleeding tendency with bleeding seen in only some individuals. This is probably because factor XI in the propagation phase may not be needed for haemostasis if adequate thrombin is generated in the amplification phase.

5. **Deficiency of von Willebrand factor (vWF)** can result in von Willebrand disease. vWF binds to subendothelial collagen and helps in platelet adhesion by binding to glycoprotein GP1b present on the platelet surface. VWF is also important for maintaining normal levels of factor VIII. VonWillebrand disease is more common when compared to haemophilias.

**Conclusion**

The cascade/waterfall model of haemostasis is useful in explaining the *in vitro* haemostatic process initiated in the laboratory. The intrinsic pathway and common pathway are tested by the aPTT and the extrinsic and common pathways are tested using prothrombin time. The *in vitro* activation of coagulation process doesn’t completely represent the global haemostatic process happening *in vivo* with interactions between tissue factor bearing cells, platelets and the intrinsic and extrinsic pathways. The *in vivo* process of haemostasis is better explained by the cell based model of haemostasis where the process is initiated in cells expressing tissue factor and propagated on the surface of platelets. This two compartment model also explains better, the underlying processes behind many of the clinical conditions due to deficiency of clotting factors.

**References**