

## Coagulation System: New Concepts for Novel Therapeutics

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### Abstract

Our understanding to the process of coagulation is based on the classic coagulation cascade, proposed in 1964 by Macfarlane and Davie & Ratnoff. While is considered to be of clinical validity, still many limitations in this model exist. Drugs for anticoagulation based in this model also show the same limitations of the model. Here we review this model and trying to find new concepts as regard this model, with aiming at finding a new way for developing new drugs for manipulating the coagulation system with more favorable profile.

### The Classical Concept of Coagulation

The classic coagulation cascade, proposed in 1964 by Macfarlane and Davie & Ratnoff is described in numerous articles and textbooks [1,2]. This proposal divides coagulation in an extrinsic pathway (involving blood elements and elements that are usually not found in the intravascular space) and an intrinsic pathway (started by components that exist in the intravascular space), which converge to a common pathway with the activation of factor X (FX). The central feature of the coagulation cascade is the sequential activation of a series of proenzymes or inactive precursor proteins (zymogens) to active enzymes, resulting in significant stepwise response amplification. The extrinsic pathway is initiated by exposure to (tissue factors) at the site of injury, so that it is sometimes referred as tissue factor pathway. In contrast, the known stimuli for intrinsic pathway are non-physiological, such as negatively charged surface celite, kaolin, or silica. In vitro the extrinsic pathway can be tested by prothrombin time (PT), while the intrinsic pathway can be stimulated by activated partial thromboplastin time (aPTT) [1,2].

From physiological point of view, coagulation system is stimulated by exposure to tissue factors at the site of injury and its interaction with factor VII, and that components of the intrinsic pathway (i.e., factors VIII, IX, XI) are responsible for amplification of this process only after a small initial amount of thrombin has been generated through the extrinsic pathway [3].

### Pitfalls in the classic concept

Deficiencies in the initial proteins in the intrinsic pathway (prekallikrein, HMWK, and factor XII) are not associated with bleeding tendencies, suggesting that the initiation portion of the intrinsic pathway (the contact phase) is not very important *in vivo* [4]. In comparison to factor XII deficiency, patients with factor XI deficiency usually have clinically significant bleeding diatheses, usually as a result of injury or surgical procedures, and especially in tissues with high fibrinolytic potential such as mucous membranes [5]. The potential explanation for the vital role of factor XI in hemostasis the ability of thrombin to back-activate factor XI, however this necessitate supra physiological level of thrombin and factor XI [6]. The classic

concept also fails to explain the well-known observation of the connection between the inflammation and thrombosis.

Accordingly, drug therapy for coagulation disorders, based on the classic concept, has many limitations, with failure of addressing the interaction between inflammation and thrombosis represents a major pitfall. So there should be another way of thinking about the process of hemostasis.

### Role of Poly -Phosphate (Poly-P)

Poly-P is a highly anionic, linear polymer of orthophosphate residues held together by high-energy phosphoanhydride bonds. It is found in all three domains of life [7]. Poly-P is often stored inside cells in complex with high concentrations of  $Ca^{2+}$ ,  $Na^+$ ,  $Zn^{2+}$ , and other cations in small, spherical, acidic, electron- dense subcellular organelles called acidocalcisomes [8]. In deed the dense granules of human platelets was discovered to be a form of acidocalcisome and was found to contain a high concentrations of Poly-P (with chain lengths of 60-100 phosphates long) [9]. Adding Poly-P can correct prolonged clot times in whole blood samples treated with heparin or direct oral anticoagulants, as well as plasma samples from patients with hemophilia A or B [10]. Poly-P can bind and activate factor XII, and this activation can be associated with release of bradykinin, accordingly the activation of the contact contact system by Poly-P can be considered to be strongly pro-inflammatory [11]. Poly-P can bind and activate factor XI and thrombin, and the interaction between factors XI, thrombin and Poly-P causes approximately 3000-fold increase in the rate of back-activation of factor XI by thrombin, allowing this reaction to occur at physiologically relevant concentrations of thrombin and factor XI [12]. Recently, platelet Poly-P was shown to enhance the rate of factor V activation to Va by factor XIa [13]. Clots formed in the presence of Poly-P are more resistant to fibrinolysis [14,15].

Poly-P can directly alter the structure of fibrin clots, increasing fiber thickness and strength, and making fibrin more difficultly for fibrinolytic enzymes to digest [16]. The observation that Poly-P induces NF- $\kappa$ B activation and leukocyte adhesion in endothelial cells and that Poly-P is secreted from activated mast cells, partially resolves the mystery of the connection between inflammation and thrombosis [17].

### Role of neutrophil extracellular traps (NETs)

Not only Poly-P, but also other physiological anionic polymers such as DNA neutrophil NETs, and extracellular RNA have been shown to trigger the contact pathway by promoting the activation of factor XII and the plasma kallikrein system [10]. Pathogens can induce neutrophils to release chromatin lined with granular components (such as myeloperoxidase [MPO], neutrophil elastase, and cathepsin G), creating fibrous nets with antimicrobial properties, capable of killing both Gram-positive and Gram-negative bacteria, collectively known as Neutrophil Extracellular Trap (NET) [17].

NETs not only entrap pathogens, they can also bind platelets and red blood cells (RBCs), thus playing a role in deep vein thrombosis (DVT) [18]. Before the link to NETs was established, nucleic acids and nuclear components were studied individually for their ability to induce coagulation. Nucleic acids activate coagulation, with RNA binding both factors XII and XI in the intrinsic pathway [19]. Also, histones increase thrombin generation in a platelet-dependent manner. Histones activate platelets, and platelet activation, in turn, promotes coagulation [20]. *In vivo*, histones likely circulate as part of nucleosomes. Intact nucleosomes/NETs promote coagulation and increase fibrin deposition [18]. The interplay of inflammation and thrombosis is well established. In coronary artery disease, MPO-DNA complexes are elevated in the more severe cases, positively associated with elevated thrombin levels, and robustly predict adverse cardiac events [21]. NETs stimulate both the extrinsic and intrinsic coagulation pathway [22]. NETs may also promote thrombolysis. *In vitro* studies have shown that NE and cathepsin G can degrade fibrin [23]. Histone Poly-P complexes can activate platelets via toll-like receptors 2 and 4, with greater potency than histones or Poly-P alone [20]. The connection between coagulation and inflammation may contribute to pathogenesis of many diseases where widespread platelet activation, thrombosis, and inflammation can cause significant mortality. Novel therapeutics targeting the coagulation-inflammation axis, therefore, has potential for treating both pathological thrombosis and thrombo-inflammatory disorders.

### The Novel Concepts: Poly Anionic Polymers

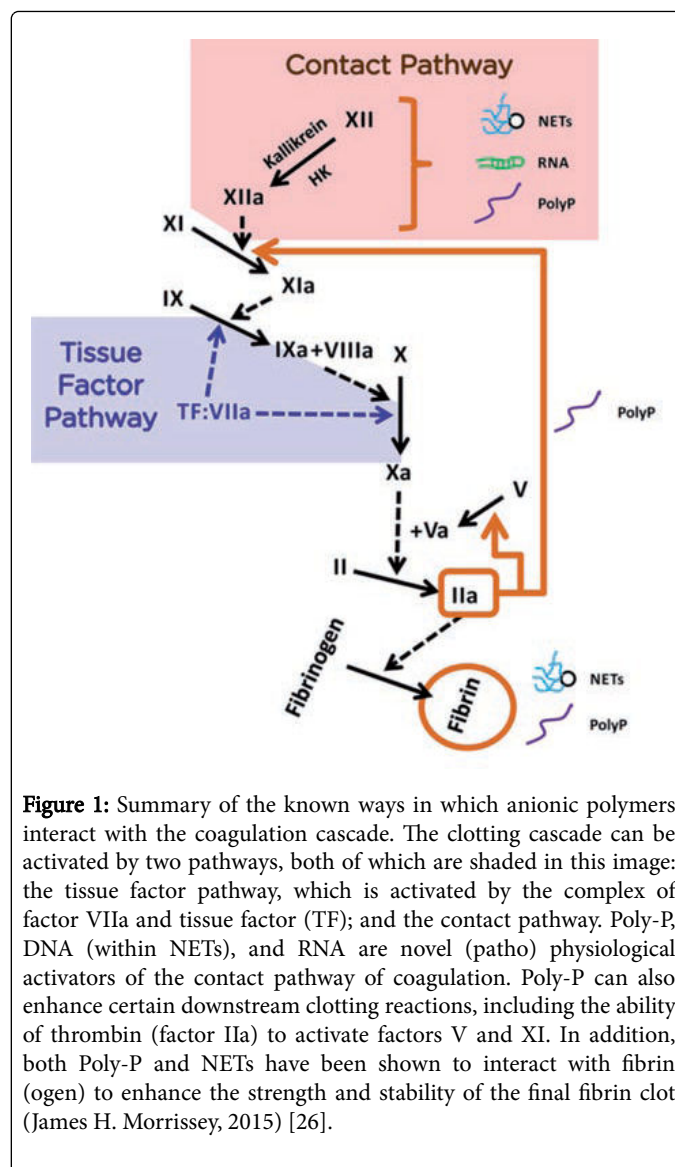
In his elegant work, James H. Morrissey, summarized all of these new concepts in the figure presented below (Figure 1). While the classic concepts of extrinsic and intrinsic pathways are still maintained, he figured out the role of poly anionic polymers in the process of hemostasis.

From this figure, we can collectively categorize the evolving roles of poly anionic polymers in to 3 stations.

Station of initiation: the poly anionic polymers, (NETs, RNA and Poly-P) can initiate the process of coagulation through activation of factor XII and release of kallikrein.

Station of consolidation: the Poly-P, specifically the platelet Poly-P is responsible for orchestrating the coagulation process, through interaction with thrombin, factor XI and factor V.

Station of stabilization: in which NETs and Poly-P stabilize the up forming fibrin polymers.



**Figure 1:** Summary of the known ways in which anionic polymers interact with the coagulation cascade. The clotting cascade can be activated by two pathways, both of which are shaded in this image: the tissue factor pathway, which is activated by the complex of factor VIIa and tissue factor (TF); and the contact pathway. Poly-P, DNA (within NETs), and RNA are novel (patho) physiological activators of the contact pathway of coagulation. Poly-P can also enhance certain downstream clotting reactions, including the ability of thrombin (factor IIa) to activate factors V and XI. In addition, both Poly-P and NETs have been shown to interact with fibrin (ogen) to enhance the strength and stability of the final fibrin clot (James H. Morrissey, 2015) [26].

### Modulating Poly-P mediated coagulation and inflammation for therapeutic benefit

Knocking down Poly-P levels in platelets in mice protects against experimentally induced thrombosis [24,25]. Using the Polyamidoamine (PAMAM) dendrimers, such as spermine, as polycationic inhibitor, Jain et al. demonstrated a promising effects in inhibiting nucleic acid- and Poly-P mediated coagulation both *in vitro* and *in vivo* [26]. The easily manipulated chemical properties of this class of dendrimer-like compounds suggest they could be a promising platform for a novel class of antithrombotic therapeutics [27]. Another approach for inhibiting anionic polymers involves infusion of enzymes to degrade the polymers before they can drive coagulation and inflammation [27]. Targeting extracellular RNA, using RNase was also proved to be of benefits in term of inhibiting coagulation, in experimental animals [28,29]. Administration of DNase has a protective effect *in vivo* in murine models of ischemic stroke, myocardial infarction, and DVT [30].

## Conclusions

The new concepts about the importance of the physiological poly-anions in the process of hemostasis are becoming facts. These new concepts cover the gaps “waterfall” or “cascade” model for coagulation that was proposed in 1964 by MacFarlane, Davie and Ratnoff [1,2]. The mechanisms of connection between inflammation and thrombosis are now clear. Our recent understanding will open the door to the possibility of novel, potentially safer therapeutics for modulating the blood clotting system.

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