EUROPEAN HEMATOLOGY ASSOCIATION

Bleeding disorders - Section 1

Biological and clinical relevance of fibrin clot structure

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Take-home messages

- Fibrin clot stability is directly influenced by the overall network structure and cellular composition.
- Patients with thromboembolic diseases exhibit abnormal clot structure.
- Shear rate alters clot composition and stability.

Introduction

Activation of the coagulation cascade ultimately results in formation of a three-dimensional cross-linked fibrin network which directly adheres to the platelet 'plug'. Thrombin cleaves fibrinopeptides from circulating fibrinogen, altering the conformational and electrostatic nature of the molecule and allowing generation of half-staggered double-stranded protofibrils. Lateral aggregation of protofibrils forms fibers (reviewed in Weisel *et al.*[1]) which are cross-linked via the action of the transglutaminase enzyme factor XIIIa. Dissolution of a clot is necessary to maintain vessel patency, failure to do so leads to occlusion (thrombosis). Fibrin degradation is attained through the action of the fibrinolytic system. The central enzyme, plasmin, is formed by activation of the circulating zymogen plasminogen by tissue plasminogen activator (tPA) or urokinase (uPA). Cleavage by plasmin releases fibrin degradation products that are readily cleared from the circulation. The visco-elastic properties of the fibrin network and its resolution are governed by numerous factors, several of which will be highlighted in this review.

Current state of the art

The structural properties of a fibrin clot, such as fiber thickness, and cellular composition, influence its stability and susceptibility to breakdown, with abnormal fibrin structure directly linked to a number of thromboembolic diseases. Clot fractal dimension (*df*), has been proposed as a potential marker for abnormal clot microstructure and predictor of recurrent venous thromboembolism.[2] The location of clot formation within the vasculature influences clot composition, with thrombi from coronary arteries being composed of thinner fibers and a higher platelet count than those from peripheral arteries.[3] Individually, thin fibrin fibers are lysed more rapidly than thick fibers, however, network composition alters this arrangement with clots composed of thick fibers being degraded faster than those of thin fibers.[4] These differences arise due to packing of these fibers, with thin fibers being more tightly packed with smaller pores, while networks of thick fibers have a looser conformation.[4] Therefore, one must consider the fibrin network as a whole when considering susceptibility to fibrinolysis. A study of lysis of single fibers indicates that susceptibility to lysis is dependent on the strain in the fiber. In response to plasmin, individual fibers either elongate or lyse transversely. Thicker fibers formed at low thrombin concentrations tend to elongate and lose the intrinsic strain assimilated during polymerization.[5]

Numerous factors are known to influence the structure of a forming fibrin network, particularly thrombin and fibrinogen. The relative levels of these proteins dictate polymerization rate, fiber thickness and porosity thereby influencing clot stability. Our work demonstrates that platelet-derived polyphosphate (polyP), a highly charged biomolecule, delays fibrin polymerization and alters the rheological and structural properties of the fibrin.[6,7] PolyP containing clots are composed of dense fibrin aggregates interspersed by pores which are resistant to tPA-mediated fibrinolysis.[7] Inhibition of platelet-derived polyP in a flow model altered fibrin structure, impeded clot retraction and conferred resistance to lysis.[8]

A number of congenital fibrinogen abnormalities exist altering both the quantity (afibrinogenemia and hypofibrinogenemia) and quality (dysfibrinogenemia and hypofibrinogenemia) of circulating fibrinogen which impacts directly on clot structure. Interestingly, patients with congenital afibrinogenemia are at high risk of arterial and venous thromboembolic events.[9] Consistently, mice lacking fibrinogen and von Willebrand fac-

tor form abundant unstable platelet-rich thrombi in arterioles that embolize readily provoking vessel occlusion.[10] These data demonstrate the need for an optimal fibrinogen concentration *in vivo* to stabilize thrombi and to scavenge free thrombin but not tip the balance towards thrombus persistence. The lack of fibrinogen may contribute to undesirable thrombotic events in these patients. The rate of fibrin polymerization and the structural features of the fibrin network help predict the clinical phenotype of patients with congenital dysfibrinogenemia. A recent study provided evidence that bleeding correlates with delayed fibrin polymerization, with clots exhibiting thinner fibers and increased permeability.[11] Fibrinogen γ', a splice variant of the fibrinogen γ-chain, interferes with fibrin polymerization leading to tightly knitted fibrin structures interspersed by large pores.[12] These effects were independent of thrombin despite the increased binding of thrombin to this variant. Recent experiments, performed under hydrated conditions, suggest that γ'-fibrinogen alters clot stiffness by changing protofibril packing.[13]

Platelets and red blood cells (RBC) influence clot structure and regulate contraction and fibrinolysis. Contracted clots are more resistance to lysis, due to extrusion of proteins and tightening of the fibrin network. New evidence demonstrates that platelets and fibrin(ogen) exert contractile forces, segregating and compressing RBCs into tightly packed polyhedrocytes within clots.[14] Interestingly, the ratio of platelets to fibrinogen influences the degree of clot contraction, with reduced contraction evident at high fibrinogen concentrations.[15] In whole blood flow models fibrin(ogen) is visualized to emanate from platelet aggregates (Figure 1). Elegant *in vivo* studies demonstrate that a dense core of highly activated platelets forms at the site of vascular injury. Fibrin and thrombin accumulate in the core of the thrombus where the rate of solute transport is low.[16,17] The core region is shrouded by a shell of loosely packed platelets displaying only transient increases in intracellular calcium with negligible degranulation.[17] This suggest

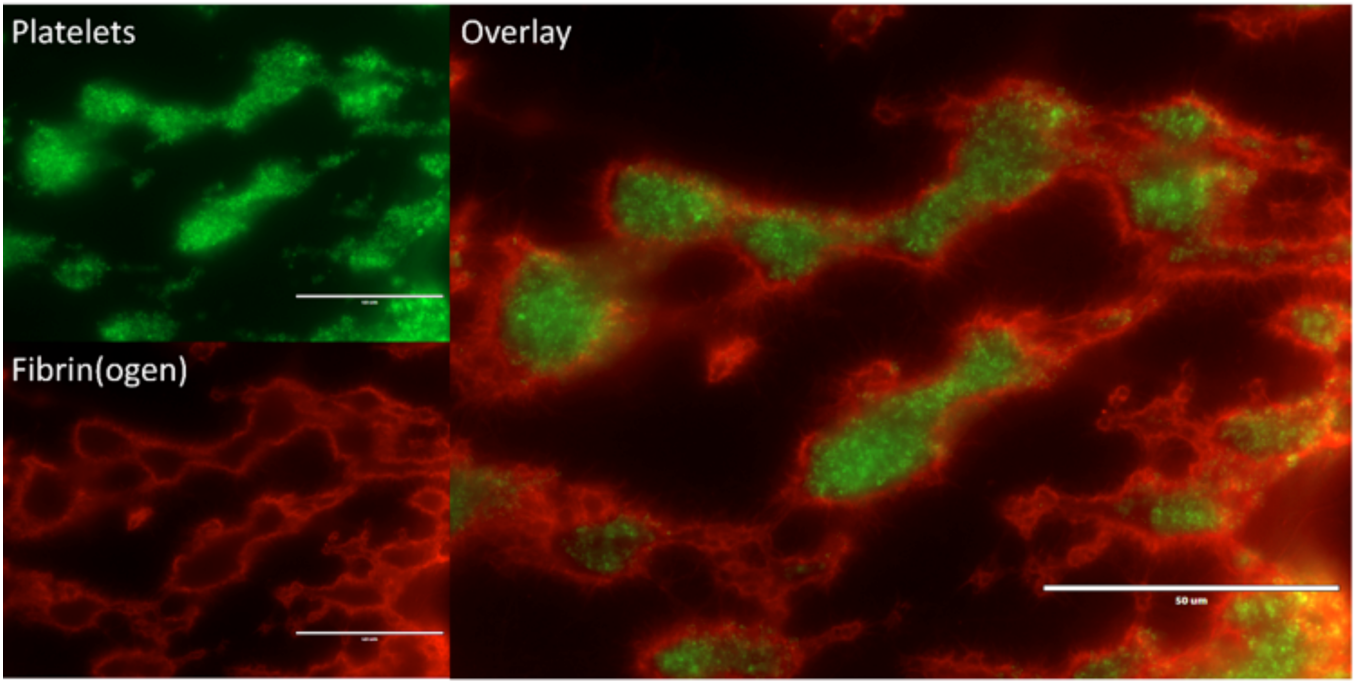

Figure 1. Thrombus formation under physiological shear rates. Platelet-fibrin thrombi were formed from whole blood. Blood containing DiOC6 (0.5 mg/mL) to label platelets (green) was re-calcified (7.5 mM CaCl2 and 3.75 mM Mg Cl_2) and perfused at 1000 s^{-1} over a collagen (100 ng)/thrombin (2 fmol)-coated surface for 6 min. HEPES buffer (10 mM Hepes (pH 7.45) 136 mM NaCl, 2.7 mM KCl, and 2 mM $MgCl_2$, 0.1% glucose and 0.1% BSA) containing fibrinogen-AF546 (75 µg/ml; red) was then perfused for 7 minutes. Images were taken on an EVOS FL imaging system with a x 60 1.42 oil immersion objective. Scale bars: 50 µm. C.S. *Whyte, G.B. Morrow and N.J. Mutch, unpublished data.*

that the granular content of platelets is largely released into the body of the thrombus, where local concentrations of platelet-derived proteins likely define the hemostatic balance. Circulating plasminogen accrues on platelet-associated fibrin, with a smaller pool directly associated with the activated platelet membrane where its local concentration will govern the rate of fibrin degradation.[18]

A large body of evidence indicates that the composition and structure of the fibrin network is modulated by shear rate, which in turn dictates susceptibility to lysis. Application of strain to fibers exposes hydrophobic residues resulting in expulsion of water and an increase in order and alignment.[19] The fibrin network primarily determines the response of forming clots to physiological stress.[20] Augmented thrombin generation under flow conditions may account for the structural changes observed in fibrin.[20] In human thrombi, internal fibrin fibers exhibit a random orientation, whereas, exterior fibers align in the direction of flow, are thinner and less porous.[21] Mechanical stretching replicates the appearance of fibers on the exterior surface of thrombi resulting in impaired fibrinolysis as a result of attenuated plasminogen activation and reduced plasmin sensitivity.[21]

Future perspectives

The composition of a thrombus is fundamental to its stability against mechanical and fibrinolytic degradation. It is now well established that the nature of the fibrin network is altered by numerous factors and that clots generated in distinct parts of the vasculature have different cellular compositions and fibrin mesh. Indeed, even within a thrombus exterior fibers exposed to shear stress may exhibit entirely different structure to those internal to the thrombus. It is clear from the literature that fibrin structure is an important determinant of normal hemostasis and inexplicitly linked to disease state, perhaps indicating that mechanisms to prevent deleterious thrombus formation should be tailored to the specific thrombus environment.

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Bleeding disorders - Section 1

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