New and Notable

Reaction Complexity of Flowing Human Blood

Scott L. Diamond
Department of Chemical Engineering, Institute for Medicine and Engineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104 USA

The coagulation pathway of blood is an ancient mechanism to stop leaks of fluid from an organism. At sites of damaged vascular endothelium, platelets and neutrophils are captured from the flow stream while tissue factor on the wall can bind factor VIIa to initiate a cascade of coagulation reactions on cellular surfaces, resulting in the conversion of prothrombin to thrombin. The thrombin cleaves fibrinogen to an active monomer that polymerizes to form a fibrin meshwork. Physiologically, convection of oxygenated blood alleviates severe diffusion limitations that would otherwise exist in the absence of flow (i.e., blood clots would cause heart attack and stroke). Despite more than a century of brilliant research in blood biochemistry, platelet and vascular wall biology, suspension rheology, and transport physics, the complexity of blood clotting under flow has prevented quantitative and predictive modeling. For example, given non-anticoagulated whole blood of a defined genotype flowing at a fixed wall shear rate over a defined reactive surface, it had been impossible to predict the instantaneous rate or final extent of platelet, neutrophil, and fibrin deposition. In this issue, Kuharsky and Fogelson (2001) have launched the first full simulation of platelet activation, deposition, and cell- and wall-dependent coagulation cascade activation during blood flow over a tissue-factor-containing surface.

By flowing isolated platelets, neutrophils, or monocytes over defined adhesive ligands attached to surfaces, many laboratories have measured the shear stress dependency of transient pausing/rolling or firm arrest. However, these studies require elimination of thrombin, which activates platelets, as well as prevention of fibrin formation, which stabilizes platelet deposits. The kinetics of the coagulation cascade assembly on activated platelets (Mann et al., 1992) and multicellular aggregation in shear flow (Laurenzi and Diamond, 1999) have both been modeled; however, these models cannot handle spatial gradients that occur during clotting on the wall.

To tackle the intertwined biochemistry, cell biology, and transport biophysics, Kuharsky and Fogelson assumed the existence of a thin, well-mixed layer near the surface. Transport of species from whole blood into this layer was then quantified by an overall mass transfer coefficient, thereby eliminating the need to solve for spatially dependent fluxes due to axial convection and radial dispersion. This insightful approach allowed the authors to solve 59 ordinary differential equations to simulate flowing human blood coagulating on a reactive wall via tissue factor initiation of the extrinsic pathway. The one catch is that the use of this well-mixed layer embeds the flow physics into the reaction kinetics. For example, the thickness h of this well-mixed layer depends on velocity. When surface densities (fmol/cm^2) are homogenized into the shell volume of height h, the new volumetric concentrations (and thus reaction rates) depend moderately on the flow velocity of the simulation.

Poised between states of flowing liquid and solid clot, the stability of blood is predicted to be balanced on the head of a pin (or, more literally, a pin-prick). Increasing the tissue factor surface density from 2 to 8 fmol/cm^2 (50 sites/μm^2) is predicted to cause a 4- to 5-order of magnitude explosion in the local thrombin concentration at physiological shear rates. Given this sensitivity, a captured neutrophil or monocyte presenting only 100 active tissue factor molecules on its surface may have important consequences in sustaining the coagulation process (Palabrica et al., 1992).

Mechanisms of tissue factor inhibition (platelet coverage versus tissue factor pathway inhibitor) were tested through the Kuharsky-Fogelson model. Hemophilias A and B provide the clinical experimental situation wherein factors VIII and IX are deficient. In order to simulate impaired coagulation after display of tissue factor to factor VIII- or IX-deficient blood, an additional physical mechanism was required—platelet deposition blocking access of blood to the tissue factor on the surface. In fact, the model predicted the increased bleeding severity of hemophilia A over hemophilia B. Importantly, the model passed an additional test in that it predicted impaired coagulation under flow for blood with reduced platelet counts, as seen in thrombocytopenia. On the opposite end of the spectrum of blood performance, a future test of the model will be its ability to predict the factor V Leiden phenotype, a clotting disorder due to a mutated factor V that is resistant to inactivation by activated protein C.

The Kuharsky-Fogelson model provides support for a fundamental insight: platelet coverage of damaged wall leads to the quenching of tissue-factor-mediated thrombosis. Although not modeled, fibrin deposits may have a similar effect if factor X must diffuse

Received for publication 17 January 2001 and in final form 17 January 2001.

Address reprint requests to Scott L. Diamond, Department of Chemical Engineering, Institute for Medicine and Engineering, 1024 Vagelos Research Laboratories, University of Pennsylvania, Philadelphia, PA 19104. Tel.: 215-573-5702; Fax: 215-573-7227; E-mail: sld@seas.upenn.edu.

0006-3495/01/03/1031/02 $2.00

through the fibrin to the tissue factor: VIIa complex at the surface. The authors support a role for the intrinsic pathway (IXa-tenase) to continue thrombosis after the first layer of platelets are deposited over the tissue factor. Blood-borne tissue factor may also serve this purpose (Giesen et al., 1999), and quantitative simulation will be useful in evaluating these issues.

Outside-in signaling alters the activation and adhesive state of the platelet. As a platelet is activated it can release ADP and thromboxane, both potent autocatalytic molecules. These interactions are captured to a first approximation through an experimentally derived rate constant for the interaction of activated platelets with unactivated platelets in a shear field. However, the magnitude of the flow will influence the local levels of these highly diffusive platelet release products. Also, the rates of successful cell-cell collisions, cell-surface collisions, and fragmentation all depend on prevailing flow conditions. When the receptor-ligand bond mechanics become better characterized, the single rate constant for platelet deposition used in the Kuharsky-Fogelson model may eventually be predicted for any set of prevailing shear rates, platelet receptor levels, and surface ligand densities.

Human blood represents a real testing ground for functional genomics. As a tissue, blood is easily obtained and has many well-defined genetic mutations leading to various bleeding or clotting phenotypes. However, blood is a tissue whose function is always dependent on the prevailing hemodynamics. Quantitative modeling of blood function under flow will have numerous diagnostic and therapeutic uses. The model of Kuharsky and Fogelson is a dramatic advancement toward the development of virtual blood, in which clotting for a genetic and pharmacological background can be simulated in an appropriate fluid mechanical context.

REFERENCES

Giesen, P. L., U. Rauch, B. Bohrmann, D. Kling, M. Roque, J. T. Fallon, J. J. Badimon, J. Himber, M. A. Riederer, and Y. Nemerson. 1999. Blood-borne tissue factor: another view of thrombosis. *Proc. Natl. Acad. Sci. USA.* 96:2311–2315.

Kuharsky, A. L., and A. L. Fogelson. 2001. Surface-mediated control of blood coagulation: the role of binding site densities and platelet deposition. *Biophys. J.* 80: 1050–1094.

Laurenzi, I. J., and S. L. Diamond. 1999. Monte Carlo simulation of the heterotypic aggregation kinetics of platelets and neutrophils. *Biophys. J.* 77:1733–1746.

Mann, K. G., S. Krishnaswamy, and J. H. Lawson. 1992. Surface-dependent hemostasis. *Semin. Hematol.* 29:213–226.

Palabrica, T., R. Lobb, B. C. Furie, M. Aronovitz, C. Benjamin, Y. M. Hsu, S. A. Sajer, and B. Furie. 1992. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. *Nature.* 359:848–851.