Computational Approaches to Studying Thrombus Development

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Abstract—In addition to descriptive biological models, many computational models have been developed for hemostasis/thrombosis that provide quantitative characterization of thrombus development. Simulations using computational models that have been developed for coagulation reactions, platelet activation, and fibrinogen assembly have been shown to be in close agreement with experimental data. Models of processes involved in hemostasis/thrombosis are being integrated to simulate the development of the thrombus simultaneously in time and space. Further development of computational approaches can provide quantitative insights leading to predictions that are not obvious from qualitative biological models. (Arterioscler Thromb Vasc Biol. 2011;31:500-505.)

Key Words: blood coagulation ■ blood flow ■ coagulation ■ platelets ■ thrombosis ■ computational model ■ stochastic multiscale model ■ thrombus development

Significant progress has been made in our understanding of the hemostatic response. For instance, coagulation pathways have been developed that describe the interactions among different elements and provide insight into the regulation of the response. Similarly, advances in platelet biology have elucidated pathways of platelet activation and identified and characterized molecular components involved in intracellular signaling, as well as surface proteins mediating adhesion to the damaged vessel wall, to other platelets, and to other thrombus components. Furthermore, the development of transgenic, gene knockout, and gene knock-in technologies has enabled exploration of the physiological roles of individual components in vivo using sophisticated hemostatic experimental systems. More recently, genomic and proteomic approaches have identified new elements modifying the hemostatic response.

The initial identification of hemostatic components and description of coagulation or platelet signaling pathways were qualitative, describing the order of interaction among components in coagulation or platelet behavior. These biological and biochemical models were extremely valuable, suggesting how these processes might be regulated and providing an understanding of how deficiencies or dysregulation of particular components leads to pathological states.

In addition to these descriptive biological models, computational models have been developed for hemostatic processes that provide quantitative characterization of thrombus development. For instance, the tissue factor (TF)-initiated coagulation model introduced by Hockin et al presented a quantitative description of the network of coagulation reactions. The model correctly predicted that there was a TF concentration threshold required to activate the coagulation system to generate the thrombin required for a hemostatic response. In addition, the computational model introduced by Purvis et al to simulate ADP-mediated platelet activation provided insight into possible mechanisms of negative-feedback signaling and cell-to-cell variation across platelet populations. Furthermore, the kinetic model of fibrin polymerization introduced by Weisel and Nagaswami revealed that changes in the rate of fibrinopeptide cleavage were sufficient to explain many nonintuitive experimental observations regarding the effects of ionic strength or reduction of fibrinopeptide B levels on fibrin polymerization.

A major challenge in our quantitative understanding of hemostasis/thrombosis is to better integrate the various subprocesses involved during clotting and thrombus development. The ability to predict how simultaneous variation of multiple hemostatic factors affects thrombus development would be of significant biomedical value. However, the challenges in developing such understandings are significant. For example, generation of thrombin on the surface of an individual platelet occurs at a subcellular nanoscale, whereas the blood flow dynamics in the vessel around the developing thrombus is described as a macroscopic process over hundreds of micrometers to millimeters. Integration of subprocesses occurring at different spatial and temporal scales is a very complex and challenging task. In addition to the technical and scientific challenges, a systems approach requires...
collaboration of researchers with very different backgrounds, including experimental biologists, biochemists, applied mathematicians, physicists, and computer scientists. Nevertheless, the potential to use simulations to predict the quantitative response of the hemostatic system to simultaneous perturbation in different subprocesses has major medical and scientific significance. For instance, identifying the concentrations or activity levels of individual components where small perturbations within normal physiological ranges have dramatic effects on the hemostatic response could identify regulatory elements and potential therapeutic targets. Determining such critical values while considering the variation of multiple other parameters in other hemostatic subprocesses is impractical in in vivo experimental systems. A systems biology approach, developing computational models to simulate these multiprocess systems, holds the promise to provide a better quantitative understanding of these complex systems.

This article provides a brief review of some of the computational models simulating hemostatic processes. These include simulations of coagulation reactions, platelet activation, platelet adhesion, and blood flow. In addition, the article describes the first attempts to integrate models of the multiple subprocesses to provide computational models of total thrombus development. (The terms clot and thrombus are used interchangeably here.) We conclude with a description of the formidable challenges that remain, as well as the potential for successful development of a systems approach to hemostasis.

Coagulation Pathway Models

In each step of the coagulation reaction cascade, generation of a protease is dependent on the action of an enzyme on a precursor molecule (zymogen). Although the components of the coagulation-anticoagulation system and the pathways describing their interactions are known, computational models that include quantitative relationships among elements are necessary to understand how the system is regulated.

In studies by Lawson et al and Jones and Mann, the first comprehensive system of ordinary differential equations (ODE) model to describe the reactions of the TF pathway was developed under the assumption of a uniformly mixed, static blood environment and unlimited supply of phospholipid. Remarkably, the model provided a good approximation of empirical data. Although the results did not yield new information about previously unknown coagulation reactions, the ability of the model to simulate the entire procoagulant pathway using specific individual rate constants provided a quantitative description of the proteolytic and catalytic events that lead to α-thrombin generation. Subsequently, an improved TF-initiated coagulation model of the extrinsic blood coagulation system was presented by Hockin et al by including blood anticoagulants (TF pathway inhibitor and antithrombin-III [AT-III]) and detailed descriptions of coagulation enzyme activities. The model accurately predicted the nonlinear dependence of thrombin generation on TF, AT-III, and TF pathway inhibitor. The model also predicted that there is a TF concentration threshold; when the TF concentration is below the threshold, thrombin production is suppressed by TF pathway inhibitor and AT-III. The value of the interplay between experiment and simulation is clearly evident in these studies. Experiments revealed the complexity of the coagulation reaction pathway and provided empirical data, and simulations enabled researchers to investigate the ability of any element to regulate the pathway at any time during the reaction. Such analyses are valuable in identifying therapeutic targets to treat pathologies associated with dysregulation of the TF-initiated coagulation pathways. Moreover, the influence of variations of concentrations of coagulation factors within the normal range that affects experimental outcomes is given in a quantitative fashion from simulations.

A kinetic Monte Carlo simulation using the Hockin et al model was introduced to accurately simulate blood coagulation with low concentrations of blood zymogens and enzymes. Monte Carlo simulations are efficient in picking up stochastic effects of coagulation factors at low concentrations that are ignored by deterministic models. Simulations revealed that the critical concentration to cause 50% of reactions containing 3-fold diluted whole blood to reach a clotting threshold of 0.05 U/mL thrombin by 1 hour. These Monte Carlo simulations help explain coagulation dynamics with a small number of TF molecules in a small volume of blood, which is difficult for experimental studies.

To take into account the significance of surface binding sites for coagulation reactions, Kuharsky and Fogelson introduced an ODE model integrating blood coagulation reactions with hydrodynamic factors and platelet interactions. The model separated coagulation reactions into those occurring on membrane surfaces of platelets or in solution phase. This model was used to compute thrombin generation and platelet binding in a thin shell above the injured wall. The model assumed that the injured vessel wall was the sole source of TF. The reactants diffused into the shell from the flowing blood or from previously bound platelets and were assumed to be homogeneously distributed within the shell. The model predicted that a threshold concentration of vascular wall TF between 2 and 20 molecules of TF/μm² was necessary to trigger blood clotting. As the thrombus developed and the reaction shell moved further from the injured vessel wall, the model predicted that TF would become limiting. The model was expanded by Fogelson and Tania to include anticoagulant factors: protein C (PC), AT-III, and TF pathway inhibitor. That study also discussed the issue that the sites where PC was activated and where PC was active were distinct, thus possibly restricting the ability of the PC pathway to limit thrombus growth.

In a study by Bungay et al, an ODE model was also proposed for the dynamics of thrombin formation in vascular and nonvascular systems that distinguished reactions on cell membranes and in bulk flow. The model assumed a uniformly mixed, static fluid environment and did not distinguish the competing roles of surface and fluid diffusion that affected the on-rates of the reactions occurring on the lipid surface. Simulations using the model demonstrated the amplification aspects of the coagulation cascade, in which the concentrations of tenase, prothrombinase, and thrombin increased by
an order of magnitude with respect to increases in lipid concentration. The model also hypothesized that lipid concentrations may influence the effectiveness of each of the inhibitory pathways. The ability of these models to describe all of the major reactions, including the binding of reactants to surfaces, provided a complete picture of thrombin generation and made these models physiologically relevant.

Luan et al. used sensitivity analysis of the network of coagulation reactions to identify fragile sites. Using parameter values reported in the literature, they identified reactions where small changes in parameter values would have dramatic effects on thrombin generation and platelet activation. This analysis identified reactions involving interactions of FX/FXa or FII/FIIa as the most sensitive to small fluctuations of relevant parameters. Interestingly, current therapeutic targets for thrombosis include FX and thrombin, consistent with the fragility analysis.

**Fibrin Network Models**

To date, only a few attempts have been made to model fibrin polymerization. Because of its complexity, a model that attempts to accurately describe fibrin polymerization has to take into account molecular dynamics for fibrinogen assembly at the molecular scale, and structures of protofibrils and fibers at the micron scale. Clearly, neither molecular dynamics nor kinetic, coarse-grained, or continuum approaches alone are able to accomplish the goal. Currently, it is still a significant computational challenge to couple these different methods. Despite these difficulties, Weisel and Nagaswami introduced a kinetic model based on understanding of fibrin assembly mechanisms that accounts for most experimental observations. The model assumed that polymerization included 3 steps: fibrinopeptide A cleavage, protofibril formation, and lateral aggregation of protofibrils to form fibers. The concentration of intermediates in fibrin polymerization, fiber diameters, fiber lengths, and protofibril lengths were computed by the model. The model predicted effects of changes in the rate of fibrinopeptide cleavage and lateral aggregation of fibers that were consistent with experimental observations.

Yang et al. developed a model of fibrinogen assembly based on crystal structures of fibrinogen and fibrin fragments. The model included 2 different knob-hole interactions, an end-to-end association by γ-chains, a lateral association by γ-chains, and a hypothetical lateral interaction between β-chains. Simulations presented evidence for coagulation proteases diffusing within a polymerizing fiber by including FXIII-mediated formation of cross-links between αC-domains. Fogelson and Keener developed a fibrin thrombus formation model and generalized the kinetic gelation equations introduced by Ziff and Ziff and Stell. The model predicted that increasing the supply rate of fibrin monomer resulted in polymerized fibrin gels with higher branch concentrations and shorter fibers connecting branch points.

**Platelet Activation Models**

There is an extensive body of experimental literature identifying components of the signal transduction pathways responding to specific platelet activators. Purvis et al. introduced a computational model using ODEs to simulate ADP-mediated activation. The model consisted of 4 signaling modules: (1) Ca\(^{2+}\) release and uptake, (2) phosphoinositide metabolism, (3) P2Y\(_1\) G-protein signaling, and (4) protein kinase C regulation of phospholipase C\(_{\beta}\). These modules were integrated into a single kinetic model. The model correctly predicted resting steady-state concentrations of Ca\(^{2+}\) and inositol 1,4,5-trisphosphate, as well as the response to ADP activation. The model has been refined using kinetic analyses to identify steady-state concentrations of components and then identifying the principal components that regulate the system.

Because platelets produce interrelated responses to combinations of signaling cues simultaneously and this is central to evaluating patient-specific clinical status, developing predictive models capable of simulating cellular response to multiple stimuli is critical. Given the complexity of an ODE model to simulate the behavior of platelets in response to a single activator (eg, modeling ADP activation used 77 reactions), Chatterjee et al. used machine training of neural networks to predict the response of platelets to multiple activators.

Neural networks are efficient in learning patterns of input and predicting outputs by altering the strength (weights) of connections in the network. Moreover, the neural network model does not require that one knows reaction rate constants, as is the case in ODE models. The system was trained using pairwise combinations of low, medium, and high concentrations of agonists for 6 receptor-mediated activation pathways. The trained network was then able to predict responses to differing combinations of multiple agonists. Furthermore, the approach successfully trained networks for platelets isolated from different patients, opening the possibility of personalizing treatments for hemorrhagic or thrombotic disorders.

**Platelet-Platelet Adhesion and Platelet–Vessel Wall Interaction Models**

Platelets adhesion to the vessel wall is an essential process in thrombogenesis. However, the process is very complicated, as it is mediated by the binding of multiple platelet receptors to 1 or more ligands. In addition, some receptor ligand interactions (GPIb–von Willebrand factor [vWF]) are dependent on shear rate, whereas the GPIb/IIa integrin receptor is modified during platelet activation, leading to changes in affinity to fibrinogen, vWF, and vitronectin. Moreover, platelet receptor–ligand interactions not only mediate platelet adhesion but also initiate intracellular signaling pathways that can result in changes in platelet shape and surface composition that affect adhesion.

Mori et al. developed a computational model of platelet-platelet binding mediated by vWF and fibrinogen interactions with receptors on adjacent platelets. The model used Stokesian dynamics to simulate simple shear flow and a Voigt model (a viscoelastic model) for binding forces between platelets mediated by vWF and fibrinogen. The simulation agreed with the general observation that thrombus development requires not only vWF but also fibrinogen.
In a series of studies, the shear-induced platelet adhesion to vWF exposed at the injured vessel was modeled. In addition, platelet-platelet adhesion mediated by GP Ibα-vWF-GP Ibα was studied. Individual platelets were modeled as a rigid oblate spheroids (or spheres). The model predicted that a platelet flowing close to the surface exhibited different dynamic characteristics and that these characteristics were affected by the platelet geometry and relative positions between platelets and the surface. In platelet-platelet adhesion, GP Ibα-vWF-A1 bond formation rate showed piecewise linear dependence on the prevailing fluid shear rate, with a sharp transition in shear at 7200 seconds⁻¹.

**Integrated Thrombogenesis Models**

With advances in computer hardware, software, and computational modeling tools, efforts have been made to integrate spatial and temporal subprocesses involved in thrombus growth, resulting in comprehensive computational models. Lobanov and Starozhilova developed 2 mathematical models simulating the effects of flow on the spatial pattern of fibrin deposition to an embolus attached to the vessel wall and on the growth of a thrombus resulting from hemorrhage into an internal space. The first model simulated embolus growth in a wall-adjacent flow region and showed that blood flow can affect the processes of blood coagulation and the structure of the thrombus. The second model was used to describe the initial stage of growth of a thrombus resulting from hemorrhage into an internal space. The model showed that growth of a thrombus depended on the blood velocity and rate of chemical reactions. Although these 2 models described fibrin deposition, they did not include platelets or a detailed description of coagulation reactions.

Anand et al coupled convection-reaction-diffusion equations with Navier-Stokes equations to describe formation and lysis of a thrombus. Blood and blood thrombus were modeled as shear-thinning viscoelastic fluids with distinct mechanical properties. Convection-reaction-diffusion equations in the model included essential constituents to describe coagulation and fibrinolysis, such as resting and activated platelets, fibrinogen and fibrin, prothrombin and thrombin, FV and FVa, FVIII and FVIIIa, FIX and FIXa, FX and FXa, tenase, prothrombinase, AT-III, PC, α₂-antitrypsin, tissue plasminogen activator (tPA), plasminogen (PLG), and plasmin (PLN). The model was used to study thrombus formation, growth, and lysis in a time-varying, fully developed, Poiseuille blood flow in a cylindrical domain. The model predicted that the shear stress was much higher in the thrombus region than in that occupied by blood, whereas across the blood-thrombus interface, the velocity, as well as the extra normal stress, did not show dramatic changes. This suggested the possibility of embolus generation at higher pressure gradients.

In studies by Wang et al and Laurenzi and Diamond, Monte Carlo simulations based on population balance equations were used to predict size and composition of heterogeneous aggregate of platelets and other blood cells. The model by Laurenzi and Diamond predicted that flowing neutrophils accelerated capture of platelets and growth of aggregates.

Models that explicitly incorporate single-platelet dynamics have been introduced recently. Pivkin et al introduced a 3-dimensional thrombus formation model. Each platelet is treated as a spherical object, and the suspension of red blood cells is treated as a continuum. Also, the model includes an ADP-induced platelet activation mechanism. Simulations using the model accurately reproduced the dependence of thrombus growth rate on blood velocity obtained in experiments. In a study by Fogelson and Guy, a microscale platelet aggregation model (as well as a continuum macroscale model) was developed. Individual platelets were modeled as a single fluid-filled closed elastic membrane immersed in a viscous fluid. This microscale model resulted in simulation of the motion of individual platelets and their interactions with each other and with surrounding fluid, and it described their response to stimuli.

**Multiscale Models of Thrombus Development**

In Xu et al, we introduced a multiscale model of thrombus development that integrated submodels of coagulation reaction, platelet behavior, and blood flow. Platelets are represented as extended objects with fluctuating boundaries by the cellular Potts model. When activated, platelets support surface-dependent coagulation reactions on their boundaries and release platelet activators. In addition, in Xu et al, the coagulation pathway model from Hockin et al was extended by distinguishing plasma-phase and platelet membrane-phase reactions controlled by platelet membrane-binding sites using the biologically relevant approach introduced by Kuhsrsky and Fogelson. A significant feature of the model is that it tracks the behavior of individual platelets and cells in space and time. Thus, it integrates discrete objects with processes best described by continuum equations. Furthermore, the model integrates processes occurring at different scales, such as the flow field around the entire thrombus, as well as coagulation reactions taking place on a platelet surface. The model predicted that low levels of FVII in blood result in a significant delay in thrombin production in venous thrombus development at an early stage.

In a study by Leiderman and Fogelson, a spatial-temporal model used coupled partial differential equations to describe multiple spatial and temporal processes, including coagulation biochemistry, activation and aggregate formation of platelets, and interaction between the blood flow and the growing thrombus. Platelets at different states were represented as concentrations. The model was used to explain the influence of the wall shear rate and near-wall enhanced platelet concentration on growing thrombi. Simulations demonstrated how wall shear rate and near-wall enhanced platelet concentrations affect the development of growing thrombi.

**Conclusion**

Combined experimental and simulation studies are starting to provide quantitative descriptions of thrombus development. Experimental studies provide quantitative data for model
refinement, validation, and verification. In turn, biologically relevant simulations using validated models have already provided new insights, as described in this review. These include processes involved in thrombus development, such as coagulation reactions,6,11,12,14 platelet signaling,7,21 fibrinogen assembly,5,16,17 and platelet-platelet and platelet–vessel wall interaction.24–28

Several advances would significantly increase the predictive power and usefulness of computational models. First, most models involving variation in space and time are 2-dimensional. Thus, it is necessary to extend models to 3 dimensions to account for realistic blood vessel geometry and blood flow. Second, the development of computationally justified approaches to couple different spatial and temporal scales would increase the power and utility of multiscale simulations. Third, closer coupling of simulation predictions with experimental results would promote the refinement and validation of computational models.

Thrombogenesis involves the complex interplay of flow dynamics, biochemical reactions, millions of cells, and the fibrin network. The development of multiscale computational models integrating these processes16–39,43 may provide valuable tools for hemostasis research. Although simulations using these multiscale models are computationally demanding, large computer clusters and parallelization algorithms make extended, complex, biologically relevant simulations possible.

Despite many challenges, the potential to simulate the quantitative response of the hemostatic system to simultaneous variations in different subprocesses has major medical and scientific significance. Such comprehensive multiscale simulations would enable one to systematically modify variable values in multiple hemostatic subprocesses to identify conditions where small changes in particular parameters have dramatic effects on thrombus development. These approaches are inconceivable in experimental systems but possible in silico with increasingly powerful computational models. Analysis of simulation results could better define complex risk factors for thrombosis or identify new therapeutic targets for hemostatic pathologies.

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References

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