Computational Approaches to Studying Thrombus Development

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Abstract

In addition to the descriptive biological models, many computational models have been developed for hemostasis/thrombosis that provide quantitative characterization of thrombus development. Simulations using computational models which have been developed for coagulation reactions, platelet activation and fibrinogen assembly were 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.

1. Introduction

Significant progress has been made in our understanding of the hemostatic response. For instance, coagulation pathways[1] have been developed that describe the interactions among different elements and provide insight into the regulation of the response. Similarly, advances in platelet biology[2] have elucidated pathways of platelet activation and identified and characterized molecular components involved in intracellular signaling as well as surface proteins mediating

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adhesion to the damaged vessel wall, to other platelets and other thrombus components. Furthermore, the development of transgenic[3, 4], gene knock-out and knock-in technologies have enabled the exploration of the physiological roles of individual components in vivo using sophisticated hemostatic experimental systems. More recently, genomic and proteomic approaches haves identified new elements modifying the hemostatic response.

The initial identification of hemostatic components and description of coagulation or platelet signaling pathways were qualitative[4, 5], 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 understanding how deficiencies or disregulation of particular components lead to pathologic 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.* [6] 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. Additionally, the computational model introduced by Purvis *et al.* [7] 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 *et al.* [19] revealed that changes in the rate of fibrinopeptide cleavage were sufficient to explain many non-intuitive experimental observations regarding the effects of ionic strength or reduction of fibrinopeptide B levels on fibrin polymerization.

A major challenge to 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 to develop such understandings are significant. As an example, generation of thrombin on the surface of an individual platelet occurs at a subcellular nanoscale while 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 background 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 sub-processes 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 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 multi process systems holds promise to provide better quantitative understanding of these complex systems.

This paper 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 paper describes the first attempts to integrate models of the multiple subprocesses to provide computational models of total thrombus development. (In the paper clot and thrombus are used interchangeably.) 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.

2. 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 which include quantitative relationships among elements are necessary to understand how the system is regulated.

In papers by Lawson and Jones *et al.* [8, 9], the first comprehensive system of an ordinary differential equations (ODE) model to describe the reactions of the tissue factor pathway was developed under 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 tissue-factor (TF)–initiated coagulation model of the extrinsic blood coagulation system was presented in Hockin *et al.* [6] by including blood anticoagulants (tissue factor pathway inhibitor (TFPI) and the antithrombin-III (AT-III)) and detailed descriptions of coagulation enzyme activities. The model accurately predicted the nonlinear dependence of thrombin generation on tissue factor, AT-III and TFPI.

The model also predicted there was a TF concentration threshold; when the TF concentration was below the threshold, thrombin production is suppressed by TFPI 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 while simulations enabled researchers to investigate the ability of any element at any time during the reaction to regulate the pathway. Such analyses are valuable in identifying therapeutic targets to treat pathologies associated with dis-regulation 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 (MC) simulation [10] using the Hockin *et al.* model [6] was introduced to simulate accurately blood coagulation with low concentrations of blood zymogens and enzymes. MC simulations are efficient in picking up stochastic effects of coagulation factors at low concentration which are ignored by deterministic models. Simulations revealed that ~0.2*pM* TF was 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 MC simulations help one understand 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 [11] 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 vessel wall tissue factor between 2 and 20 molecules of TF /μm^{-2} 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 in Tanya and Fogelson [12] to include anticoagulant factors; Protein C, ATIII and TFPI. The later paper [12] 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 paper by Bungay *et al.* [13] an ODE model was also proposed for the dynamics of thrombin formation in vascular and non-vascular systems which 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 [14]. 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.

3. Fibrin network models

Up to date, only a few attempts have been made to model fibrin polymerization. Due to its complexity, a model which 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 or 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 *et al.* [15] introduced a kinetic model based on understanding of fibrin assembly mechanisms that accounts for most experimental observations. The model assumed that polymerization included three steps: fibrinopeptide A cleavage, protofibril formation and lateral aggregation of protofibrils to form fibers. The concentration of intermediates in fibrin polymerization, and fiber diameters, fiber 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 which were consistent with experimental observations.

Yang *et al.* [16] developed a model of fibrinogen assembly based on crystal structures of fibrinogen and fibrin fragments. The model included two 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 factor XIII mediated formation of crosslinks between αC-domains. Fogelson *et al.* [17] developed a fibrin thrombus formation model and generalized the kinetic gelation equations introduced by Ziff *et al.* [18, 19]. 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.

4. Platelet activation models

There is extensive experimental literature identifying components of the signal transduction pathways responding to specific platelet activators. Purvis *et al.* introduced a computational model [7] using ODEs to simulate ADP mediated activation. The model consisted of four 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 Cβ that were integrated into a single kinetic model. The model correctly predicted resting steady state concentrations of Ca^{++} 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 [20].

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 simulate 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 (e.g., modeling ADP activation utilized 77 reactions [7]), Chatterjee *et al.* used machine training of neural networks to predict the response of platelets to multiple activators [21]. 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 avoids complexities such as knowing reaction rate constant as in ODE models. The system was trained using pairwise combinations of low, middle 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.

5. Platelet-platelet adhesion and platelet-vessel wall interaction models

Platelets adhesion to the vessel wall is an essential process in thrombogenensis. However, the process is very complicated as it is mediated by the binding of multiple platelet receptors to one or more ligands. Additionally, some receptor ligand interactions (gpIb – von Willebrand factor (vWF)) are dependent on shear rate while the gpIIbIIIa integrin receptor is modified during platelet activation leading to changes in affinity to fibrin(ogen), vWF and vitronectin[22, 23]. 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.* [24] developed a computational model of platelet-platelet binding mediated by vWF and fibrinogen interactions with receptors on adjacent platelets. The model uses Stokesian dynamics to simulate simple shear flow and a Voigt model (a visco-elastic model) for binding forces between platelets mediated by vWF and fibrinogen. The simulation agrees with the general observation that thrombus development requires not only vWF but also fibrinogen.

In a sequence of papers [25-28], the shear-induced platelet adhesion to vWF exposed at the injured vessel was modeled. Additionally, platelet-platelet adhesion mediated by GPIba-vWF-GPIba 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 dynamical characteristics and these characteristics were affected by the platelet geometry and relative positions between platelets and the surface. In platelet-platelet adhesion, GPIba-vWF-A1 bond

formation rate was piecewise linear dependence on the prevailing fluid shear rate, with a sharp transition in shear at $7200s^{-1}$.

6. Integrated thrombogenensis models

With the advancement 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 *et al.* [29] developed two mathematical models were developed 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. While these two models described fibrin deposition, they did not include platelets or a detailed description of coagulation reactions.

Anand *et al.* [30] 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, PPC, α_1-antitrypsin, tPA and PLS and PLA. The model was used to study thrombus formation, growth and lysis in a time varying, fully developed, Poiseulille 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 possibility of embolus generation at higher pressure gradients.

In papers by Wang *et al.* [31] and Laurenzi *et al.* [32], Monte Carlo simulations based on population balance equations were employed to predict size and composition of heterogeneous aggregate of platelets and other blood cells. The model by Laurenzi *et al.* [32] predicted that flowing neutrophils accelerated capture of platelets and growth of aggregates.

Models which explicitly incorporated single platelet dynamics have been introduced recently. Pivkin *et al.* [33] introduced a three-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 [34]. In paper by Fogelson *et al.* [35], 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 motion of individual platelets, their interactions with each other and surrounding fluid and described their response to stimuli.

7. Multiscale models of thrombus development

In papers Xu *et al.* [36-39], we introduced a multiscale model of thrombus development which integrated submodels of coagulation reaction, platelet behavior and blood flow. Platelets are represented as extended objects with fluctuating boundaries by the Cellular Potts Model (CPM) [40-42]. When activated, platelets support surface dependent coagulation reactions on their boundaries and release platelet activators. In addition, in Xu *et al.* [38] the coagulation pathway model from [6] was extended by distinguishing plasma-phase and platelet membrane-phase reactions controlled by platelet membrane-binding sites using the biologically relevant approach introduced in [11]. A significant feature of the model is that it tracked the behavior of individual platelets and cells in space and time. Thus it integrated discrete objects with processes best described by continuum equations [36, 37]. Furthermore, the model integrated 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 factor VII in blood resulted in a significant delay in thrombin production in venous thrombus development at an early stage.

In paper by Leiderman and Fogelson [43], a spatial-temporal model utilized coupled partial differential equations to describe multiple spatial and temporal processes including coagulation biochemistry [11], 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.

8. Concluding remarks

Combined experimental and simulation studies are starting to provide quantitative descriptions of thrombus development. Experimental studies provided quantitative data for model refinement, validation and verification. In return, biologically relevant simulations using validated models already provided new insights described in this review. These include processes involved in thrombus development such as coagulation reactions [6, 10, 11, 13], platelet signaling [7, 21], fibrinogen assembly [15-17] , platelet-platelet and platelet-vessel wall interaction [24-28].

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

Thrombogenensis involves the complex interplay between flow dynamics, biochemical reactions, millions of cells and the fibrin network. The development of multiscale computational models integrating these processes [36-39, 43] may provide valuable tools for hemostasis research. While simulations utilizing 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. Such 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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