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INVITED REVIEW

Systems biology to predict blood function

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Summary. Systems biology seeks to provide a quantitative framework to understand blood as a reactive biological fluid whose function is dictated by prevailing haemodynamics, vessel wall characteristics, platelet metabolism, numerous coagulation factors in plasma, and small molecules released during thrombosis. The hierarchical nature of thrombosis requires analysis of adhesive bond dynamics of activated platelets captured from a flow field to a growing thrombus boundary along with the simultaneous assembly of the coagulation pathway. Several kinetic models of protease cascades have been developed. A full bottom-up model of platelet intracellular metabolism is now available to simulate the metabolism of resting platelets and platelets exposed to activators. Monte Carlo algorithms can finally accommodate platelet reaction, dispersion, and convection for full simulation of platelet deposition and clotting under flow. For clinical applications, the systems biology prediction of patient-specific pharmacological response requires the final assembly of platelet intracellular metabolism models with coagulation protease network models.

Keywords: adhesion, convection, diffusion, shear stress, simulation, thrombosis.

Introduction

Human blood presents itself as an ideal multicellular 'organ' for systems biology analysis. Blood is amenable to experimentation via high throughput liquid handling and microfluidics. G-protein coupled receptor (GPCR) signaling and outside-in/inside-out kinetic networks are fully independent of changes in transcription in the anuclear platelet. Protease networks and kinetics are largely defined for coagulation, although important uncertainties remain where kinetic modeling can help test and explore reaction scenarios for plausibility. The human plasma and platelet proteomes are continually undergoing increased refinement, and the platelet transcriptome has about ~3000 detectable mRNAs.

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A systems biology model of platelet and plasma function seeks to provide a realistic description of intracellular signaling, platelet membrane receptor function, and assembly of extracellular processes on platelet membranes or in plasma. Essential to blood systems biology is the spatial-temporal prediction of blood function under various haemodynamic conditions that regulate adhesive bond function and concentration of local species. As a result of complexity in systems biology, *coarse-graining* is the act of integrating or averaging over short time scale information, short length scale information, or reaction complexity to obtain simpler and less computationally intensive descriptions of a process (e.g. Michaelis–Menten kinetics coarse-grains over the exact reaction mechanisms). The multiscale and hierarchical nature of thrombosis is now reviewed with respect to: (i) hemodynamics; (ii) bond mechanics; (iii) platelet signaling; (iv) coagulation kinetics; and (v) platelet aggregation and thrombosis under flow.

Haemodynamics

The macroscopic characteristics of blood flow as a two-phase fluid influence platelet adhesion dynamics and clot stability during thrombosis. Local wall shear rate dictates how rapidly cells encounter the wall or one another, and how rapidly soluble mediators are delivered or removed from a local region of thrombosis. Also, the prevailing wall shear stress dictates the force on adhesive bonds. Important biophysical attributes of blood flow include the migration of erythrocytes to the center of the flow (resulting in a blunted flow) and the consequent drift of platelets towards the platelet rich layer near the wall where platelet concentrations are ~3- to 6-fold higher than in blood. Platelet migration has been studied [1] and is important to thrombosis simulations; however, generalized equations relating the platelet drift velocity to wall shear rate and haematocrit are only now within reach by computer simulation [2]. While systems biology approaches tend to be molecularly focused, macroscopic and mesoscale flow physics controls platelet flux and bonding to the surface.

Bond mechanics

A critical threshold level of flow ('hydrodynamic thresholding') at a wall shear rate, $\gamma_w \sim 100\ s^{-1}$ (wall shear stress of $\tau_w \sim 1$ dyne cm^{-2}) is required for platelet GPIbα to bind to von

Willebrand factor (vWF) [3,4]. The Bell model is an empirically useful approach for characterizing force sensitivity of bond lifetimes: $k_{off} = k_{off}(0) \exp(rF_B/k_BT)$ where k_{off} is the dissociation constant, F_B is the force on the bond, $k_{off}(0)$ is the unstressed off-rate, r is empirically measured reactive compliance, and k_B and T are the Boltzmann constant and temperature, respectively. Bell model parameters have been measured for selectins, GPIbα/vWF ($k_{off} = 3.45\ s^{-1}$, r = 0.016 nm for wildtype A1 domain), and fibrinogen/$\alpha_{IIb}\beta_3$ in a variety of ways by a number of labs. The GPIb/vWF bond displays slip bonding ($r > 0$) at forces above ~5 to 25 pN [3,4]. Recently, a catch bonding regime has been detected by atomic force microscopy at low forces below ~25 pN [5]. Importantly, these descriptions of single bond dynamics (such as the Bell model, Dembo model, catch-slip model, etc.) are purely phenomenological and average over the numerous atomic level interactions in a force-loaded active site. Less computational effort has yet been applied to tackle the complexity of platelet capture, deformation, activation, and firm arrest as the platelet utilizes abundant copies of receptors such as GPIbα, GPVI, $\alpha_2\beta_1$, $\alpha_{2b}\beta_3$, and CD36 to interact with damaged vessel wall or a growing thrombus. Lacking direct calculations, the sticking probability is a useful context-dependent, coarse-grained description of these numerous molecular-level and atomic-level sub-processes.

Platelet signaling

Activators of platelets such as collagen, thrombin, thromboxane, and ADP trigger mobilization of intracellular calcium. Purvis *et al.* [6,7] developed a molecularly detailed, bottom-up ordinary differential equation (ODE) model of platelet $P2Y_1$ signaling to include phosphoinositide and calcium regulation. Bottom-up models allow the exploration of precise molecular mechanisms. The full model (five compartments, 77 reactions, 132 fixed kinetic rate constants from literature, and 70 species) comprises four interlinked kinetic modules to accommodate calcium release, GPCR signaling, phosphoinositide metabolism, and PKC feedback regulation of phospholipase-β (Fig. 1A). This model does not yet consider store operated calcium entry, the acidic calcium store, or $P2Y_{12}$ function. The model accurately predicted: (i) steady-state resting concentrations for Ca_i, IP_3, DAG, phosphatidic acid, PI, PIP, and PIP_2; (ii) transient increases in intracellular calcium (Fig. 1), IP_3, and Gq-GTP in response to ADP; and (iii) the volume of the platelet dense tubular system (0.5–5% of total volume). The model required the platelet to have very high ratios of SERCA/IP_3-receptor, consistent with the fact that SERCA-3 mRNA is an abundant and platelet-specific transcript. Using stochastic simulation of single platelets, the model accurately predicted the broad frequency distribution of measured spiking events and demonstrated that asynchronous spiking was a consequence of stochastics resulting from the small volume of the platelet. These noisy spiking characteristics were lost when the volume of the platelet was raised artificially in the simulation. The initial condition for the 70 species is largely unknown, although certain species are known in resting platelets, and the remaining species were thus highly constrained by these known values, reaction network topology, and the homeostasis constraint [7].

Coagulation kinetics

Blood coagulation is a platelet surface-catalyzed protease cascade with autocatalytic amplification and multiple layers of inhibition. There is a striking sensitivity of coagulation to initial conditions of picomolar tissue factor (TF) levels. From a systems biology perspective, modeling can help address central questions regarding: (i) the regulation of response to perturbation; (ii) the dynamic stability of blood *in vivo* or *ex vivo* in the face of zero or near-zero levels of circulating TF or active proteases; and the (iii) safe pharmacological alteration of blood function. The Hockin–Mann model [8] is a pseudohomogeneous ODE model that assumes a fully active platelet at time equals zero. This model makes very accurate prediction of clotting times as [TF] is increased from 1 to 10 pM. However, the model predicts an infinite clotting time if [TF] < 1 pM and cannot accommodate the role of added platelet activators that accelerate clotting rates in CTI-treated whole blood [9]. A coarse-grained platelet can be added to the Hockin–Mann Model (Fig. 1B) by requiring off-rates to decrease due to anionic lipid exposure as the platelet activates (ε increases from 0.01 to 1 as thrombin increases from 0.01 to 0.1 U mL^{-1}). By correcting various kinetic constants in the Hockin–Mann model with $k_{platelet} = (\varepsilon/\eta)\ k$, where η is a constant from 0.01 to 1 to adjust for the platelet context or parameter uncertainty and by including a TF source term = 0.1 molecules/platelet per min, it is possible to predict a full TF titration of CTI-treated blood (Fig. 1D). Simulations with initial low levels of factor XIa (but no TF source term) do not satisfy the experimental data shown in Fig. 1D for [TF] < 1 pM. Addition of a XIIa or a Xa source term instead of a TF source term also correct model predictions in the absence of added TF.

The Hockin–Mann model and the coarse-grained Platelet–Plasma model (Fig. 1B) fail to predict situations where platelet concentrations increase dramatically relative to plasma, such as at the site of deposition on the wall. A heterogeneous model explicitly accounts for the changing platelet surface and platelet number and allows species concentrations to be defined on the platelet and in the fluid. This was implemented by Kuharsky–Fogelson [10] in a remarkable ODE model of platelet deposition, activation, and mass transfer of reactive species. Varner *et al.* [11] further explored the dynamical sensitivities of a Fogelson-type model to identify 'fragile' points in the cascade that correlate well with druggable targets pursued in the clinic.

Platelet aggregation and thrombosis under flow

Isotropic bulk aggregation experiments in cone-and-plate viscometers provide sticking probabilities for activated platelets, and these values are easily accommodated in population balance models [12] or in Monte Carlo simulations based on

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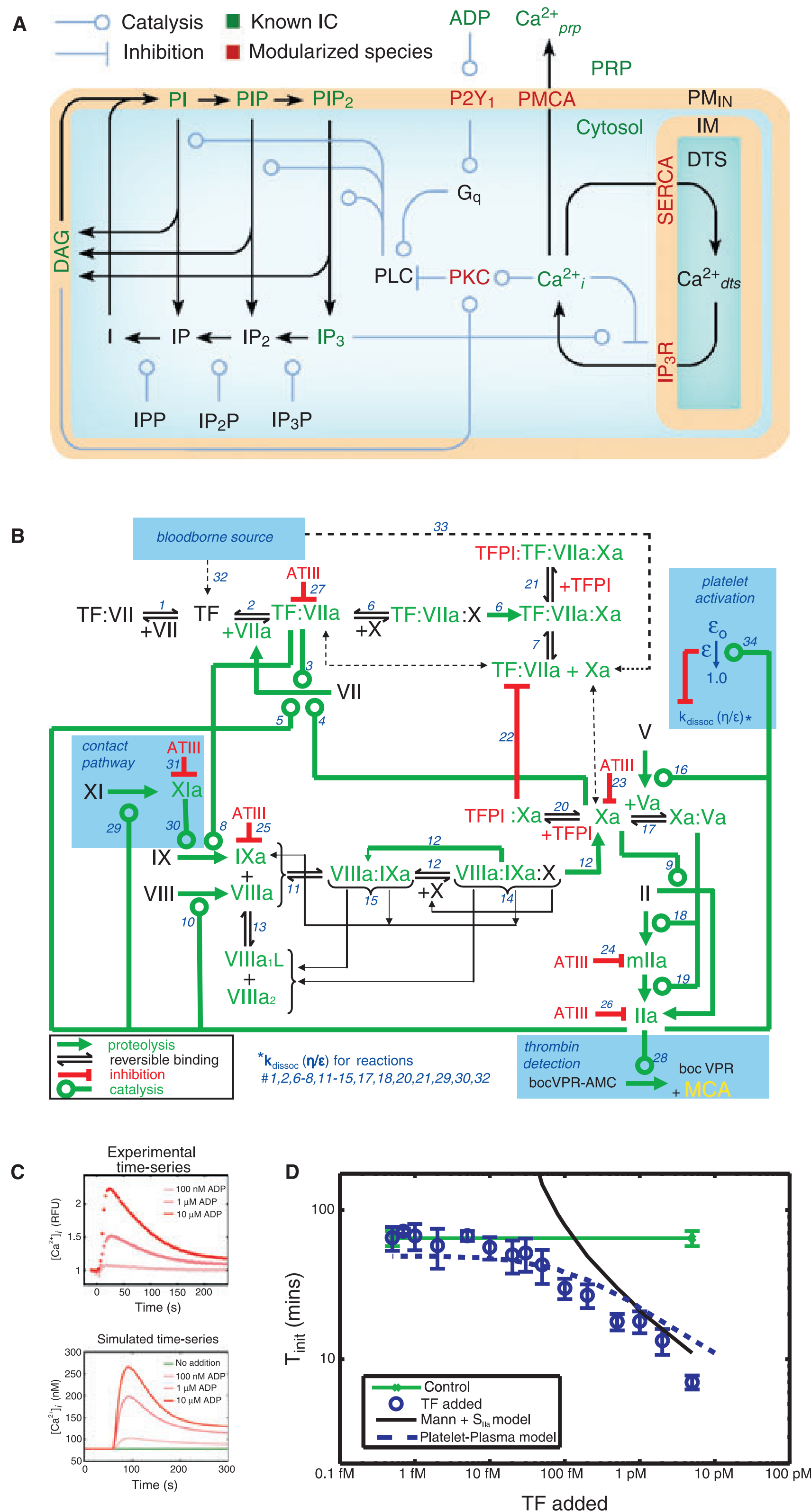

Fig. 1. A systems biology model that allows the platelet to maintain stable levels of IP_3 and Ca_i during resting conditions, but also allows dose-dependent calcium mobilization when $P2Y_1$ is activated with ADP (A). Coarse-grained 'Platelet–Plasma' model for coagulation that accounts for thrombin-dependent activation of platelets ($\varepsilon = 0.01$–1 as thrombin increases), which in turn enhances coagulation factor binding by reducing the dissociation rate (B). Experimental data and simulation for platelet calcium mobilization following addition of ADP (C). Clotting initiation time data obtained as in [9] for CTI-treated whole blood are accurately modeled by inclusion of a TF source term of 0.1 molecules per platelet per min to the 'Platelet–Plasma' model, while the Hockin–Mann model [8] accurately predicts clotting for [TF] > 1 pM (D).

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the Gillespie Method [13]. For deposition on the wall, the modeling is far more complicated. Several mathematical treatments of thrombosis have been developed to include various effects of flow. By imposing a thrombus growth rate and flux of ADP, thromboxane A_2, and thrombin, Folie and McIntire [14] performed a computational study to simulate the concentration profiles of released platelet activating factors, as they dilute in a flow field. The model predicted an active cloud of these compounds around the thrombus, and the size of this cloud decreases with increasing shear rate. Ideally, models are sought that predict, not impose, the thrombus growth rate. More recently, Kuharsky and Fogelson [10] developed a full transport-reaction coagulation model that takes into account surface dependent reactions, transport of chemicals and cells due to flow, and populations of resting and activated platelets. In the Kuharsky–Fogelson model, convection and diffusion processes were treated with a mass transfer coefficient between the bulk blood compartment and a well mixed, thin and small (10×10 μm) compartment near the surface, thereby converting a difficult multicomponent convection-diffusion problem into a transient problem, where spatial gradients are not needed or calculated. The model predictions of threshold concentrations of surface TF between 2 and 20 molecules TF μm^{-2} to trigger clotting from 100 to 1000 s^{-1} have been verified experimentally [15]. The Fogelson Lab has also developed continuum models for the growth of thrombus in stenosis and the deposition of fibrin under flow. Pivkin *et al.* [16] used a force-coupling method and a coarse-grained, three-state platelet model (passive, triggered, activated) and adhesion process to predict biphasic thrombus growth rate as flow was increased. Critical to these simulations was the interaction time of a platelet near the thrombus to predict the prevailing shape of the thrombus. Flamm *et al.* [17] recently published a full lattice kinetic Monte Carlo solver for convection-diffusion of reactive particles, and this allows computationally feasible simulation of platelet deposition with a changing flow field, where platelet metabolism and coagulation cascade assembly can be fully solved in space and time.

Conclusions

The combination of simulation and experiment to understand the factors governing thrombus growth and stability is central to the goals of blood systems biology. Quantitative factors governing clot stability, intrathrombic signaling, and intrathrombic assembly of fibrin remain poorly resolved. Future challenges or tests of blood systems biology models require: (i) prediction of blood clotting times, when known amounts of platelet modulators or coagulation factors are added to minimally perturbed blood; and (ii) prediction of clot formation rates and composition for a defined flow rate and defined surface and sensitivity to pharmacological perturbation. Systems biology, when coupled with measurements of patient-specific blood response, would be applicable in identifying undefined blood defects and/or response to pharmaceutical interventions.

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Disclosure of Conflicts of Interests

Author declares no conflict of interest.

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