MODEL CREDIBILITY PLAN

Title: **Multiscale modeling and empirical study of a mechanism limiting blood clot growth (NIH U01-HL116330)**

Mark Alber, UC Riverside, CA (PI)
John Weisel, University of Pennsylvania, PA
Zhiliang Xu, University of Notre Dame, IN
Danny Chen, University of Notre Dame, IN

The proposal combines development of 3D Multiscale Blood Clot Modeling Environment (MBCME-3D) and coupling MBCME-3D simulations and specifically designed experiments using optical tweezers and microfluidic chambers, to study two specific roles that a fibrin network plays in regulating blood clot growth: 1) impeding protein transport; and 2) mediating platelet-fibrin binding kinetics under physiological or pathological conditions. This will result in detailed examination of the common clinical scenario of increasing blood shear in response to partial obstruction and narrowing of the vessel lumen, which is considered a critically important component, affecting both the generation of fibrin and binding of platelets, the mechanisms determining blood clot growth and its stability.

1. List of Planned Actions

Year 1, 2: Image analysis algorithms and multiscale submodels of diffusion of hemostatic factors, subcellular element submodels of fibrin networks and platelets have been developed and verified.
Year 3, 4: The blood factor and cell transport submodels were developed. Platelet association rate constant were defined based on calibrated simulations and experiments and the submodel of platelet adhesion to a fibrin network surface was developed. All the submodels were compared and validated with experiments.
Year 5: Coupling of parallelized submodels on CPUs and GPUs will be implemented. The MBCME-3D computer package will be released and the feedback through the MSM workshops and Wikis will be collected. Predictive simulations will be performed and compared to microfluidic chamber experiments and data available in the literature.

2. Information Gained by Each Credibility Action

Development, verification and evaluation of the 3D Multiscale Blood Clot Modeling Environment (MBCME-3D): This action ensures that the model simulation results are consistent with the outcomes provided by theoretical analysis of platelet flipping under shear and protein diffusivity as well as with available experimental data on fibrin network structural and mechanical properties and platelet-fibrin interactions. C++ and MATLAB codes are being modularized and combined. GUI module and documentation for user-friendly usage and modifications of the MBCME-3D is being finalized. The MBCME-3D is being evaluated by three outside groups: Dr. Alison Marsden's group from Stanford University School of Medicine, Dr. Xiao Yun Xu's group from the Department of Chemical Engineering, Imperial College London, UK, as well as Dr. Andrew Kahn's lab from the Altman Clinical and Translational Research Institute (ACTRI), University of California San Diego School of Medicine.

Optimization of the 3D Multiscale Blood Clot Modeling Environment (MBCME-3D): This action ensures that the parallelized code implementing coupled submodels works smoothly and that the package can be successfully transferred to and ran on high-performance GPU and CPU cluster platforms to achieve best performance. These codes are also being modularized for easy maintenance and modification.

3. Actions and Activities Classified within the CPMS TSR Framework

Number	Rules	Actions/Activities
1	Define context clearly	Multiscale model simulations of fibrin network mechanical and structural changes combined with platelet-fibrin interactions are used to predict clot behavior under specific blood flow conditions.

2	Use appropriate data	Most of the experimental and theoretical data used for model development and validation have been either obtained from our own experiments (see [Kim et al 2017a, Hook 2017b, Kim et al 2016, Kim et al. 2014] and papers published previously by our group).
3	Evaluate within context	The multi-scale model is being evaluated for its ability to produce metrics characterizing patient specific blood clot emboli generation mechanisms and clot mechano-structural responses to external forces using obtained experimental data. Model evaluation has been performed based on ensemble averaging and experimental data distributions of fibrin network structural properties (length, thickness, connectivity) (Kim et al 2011, Kim et al 2014, Kim et al 2016) to simulate mechanical responses and structural properties of fibrin clots as well as transport properties of the networks ([Kim et al 2016, Britton et al 2018]). Stress-strain dependences, individual fiber deformations and reorientation, blood factor mobility within the network, $\alpha_{2b}\beta_3$-mediated platelet-fibrin interactions (binding-unbinding rates) and fibrin deformations have been evaluated. The data obtained in [Kim et al 2017a, Kim et al 2014] including the rate of platelet filopodia contraction, individual fibrin fiber deformations and properties, fibrin compactization, kinetics of platelet contractile action under physiological conditions have been used for calibration and validation of fibrin network model deformation and the platelet-fibrin model.
4	List limitations explicitly	Model simplifying assumptions and associated model limitations include lack of detailed representation of fibrin polymerization and the inability of the model at this stage to predict fibrin network dynamics at the molecular scale.
5	User version control	We have not yet made any of the models developed in this project public, but we do use at this time internal version control by using Git or CVS.
6	Document adequately	The model simulation codes are fully documented and are available at: https://www3.nd.edu/~zxu2/blood_clot_proj.html
7	Disseminate broadly	We will provide model and image analysis packages, simulation scenarios, representative results and experimental datasets against which the model was tested, for the MSM evaluation and will made them publicly available through both GitHub and website: https://www3.nd.edu/~zxu2/blood_clot_proj.html.
8	Get independent reviews	All our model simulation results and the codes are being evaluated evaluated by a third-party experts in multiscale modeling of blood clotting and medical applications including: Dr. Alison Marsden's group from Stanford University School of Medicine, Dr. Xiao Yun Xu's group from the Department of Chemical Engineering, Imperial College London, UK, as well as Dr. Andrew Kahn's lab from the Altman Clinical and Translational Research Institute (ACTRI), University of California San Diego School of Medicine

9	Test competing implementations	We have evaluated codes on single and multiple processors including CPUs and GPUs clusters to test for consistency of the results and to provide optimal system configurations to users.
10	Conform to standards	We have adopted generally accepted standards for blood clotting modeling and code implementaion.

How Planned Activities Will Lead to a Credible Model: Acquiring data in experiments specifically designed for model development, calibration and validation, a necessary important part of the project, is expected to result in a multiscale model successfully reproducing the key metrics of human blood clot deformation, mechanics, and stability. The model is expected to account for the impact of various biological and physical factors on the developed clot behavior taken from our experiments and literature including variable flow shear rates, platelet count, platelet-fibrin interaction, platelet activation level, fibrin network structure and mechanics, and the presence of deformable red blood cells. In model derivation, each submodel will carefully incorporate known biological and physical mechanisms and be ensured to obey laws of physics. Model parameter values will be estimated statistically.

Model validation and verification will be performed by comparing with experiments. Specifically, platelet-fibrin binding-unbinding force measurements obtained using experiments with optical tweezers, and distribution of blood factors transporting through the fibrin clot has been used to validate binding kinetic model (Hook et al. 2017), and protein diffusion model [Kim et al. 2013]. Imaging data from z-stacks of platelet-rich-plasma clots obtained in experiments at various levels of contraction and compression will be used to validate the extended continuous and multiscale models developed for studying clot contraction and red blood cell (RBCs) compression in a clot. The credibility of the model will be demonstrated and confirmed by providing fully documented and tested in detail models to external evaluators and MSM community.

Progress to-date: a) We have developed new modeling and computational methodologies for detailed studying of blood clots at various spatial and time scales (Xu et al. 2018, Xu et al. 2017, Wu et al. 2014, Hook et al. 2017, Bukac and Alber 2017, Kim et al 2013). b) Using state-of-the art experimental methods we have quantified structural mechanical response of fibrin networks to compression and discovered a mechanism of platelet-fibrin interactions leading to contraction of the entire clot ([Kim et al 2017a, Kim et al 2017b, Hook et al. 2017, Kim et al. 2016, Kim et al 2014]). The data obtained in these experiments is used for our model calibration and validation. c) CPU and GPU simulations were ran on high-performance supercomputers at University of Norte Dame and Argonne National Lab to calibrate individual submodels and multiscale model. d) Multiple scales in the multi-scale model were coupled from the receptor- to cell- to clot levels ([Xu et al. 2017, Wu et al. 2017, Bukac and Alber 2017]). d) A multi-phase complex fluid model derived by using phase-field and energy variation methods and energy stable numerical scheme for solving model equations are introduced in (Xu et al. 2018). This model can simulate mechanical responses of a class of gel-type fluids mixed with biological cells. e) Fibrin network model being implemented on GPUs will be coupled with our previously introduced platelet model (Wu et al. 2014) for investigating platelet-fibrin interaction leading to blood clot contraction. Structures of platelets and fibrin network will be modeled explicitly, namely, they will be represented by mesh surfaces and network with links and nodes. Fibrin-platelet binding kinetics will be described by using our kinetic model developed in [Hook et al. 2017].

Plans for the next reporting cycle: a) Calibrate and evaluate recently developed fibrin clot submodel [Britton et al 2018], accounting for fiber-fiber cohesive interactions, an important process promoting clot stiffening upon stretching. After calibration using experimental data for mall-size clots, the code will be parallelized, and model simulations will be run on GPU computer clusters to incorporate realistic structures of fibrin networks and clot sizes. The model will be validated against experimental data (Brown et al. 2009, Gersh et al 2010). b) Combine continuum multiphase model with microscale cell-based SCE models of individual platelets and fibrin networks to calibrate macroscale parameter values and to run predictive simulations at different scales. c) Use developed combined model for studying blood clots being compressed by platelets pulling on fibrin network and calibrate and validate the model using our experimental data characterizing platelet-fibrin interactions [Kim et al, 2017a, b]. d) Perform experiments elucidating different aspects of platelet-fibrin molecular interaction and platelet survival as well as platelet receptor conformational changes and study their impact on the entire clot platelet-induced deformation. Use obtained experimental data for

further calibration of the whole multi-scale model and testing specific model predictions. e) Develop and implement new submodel of red blood cell (RBCs) compression, which will be calibrated by using our experimental data [Tutwiler 2015] characterizing RBCs in blood clot compressed by platelets pulling on fibrin network.

References

1. Xu S, Xu Z, Kim O.V., Litvinov RI, Weisel JW, Alber M. (2017) Model predictions of deformation, embolization and permeability of partially obstructive blood clots under variable shear flow. *Journal of the Royal Society Interface*. Nov 1;14(136):20170441.
2. Kim O.V., Litvinov R.I., Alber M.S., Weisel J.W. (2017a) Quantitative structural mechanobiology of platelet-driven blood clot contraction. *Nature Communications*, Nov 2.
3. Höök P, Litvinov RI, Kim O.V., Xu S, Xu Z, Bennett JS, Alber MS, Weisel JW. (2017) Strong Binding of Platelet Integrin αIIbβ3 to Fibrin Clots: Potential Target to Destabilize Thrombi. *Scientific Reports*. 2017 Oct 11;7(1):13001.
4. Bukač, M. and Alber, M., 2017. Multi-component model of intramural hematoma. *Journal of biomechanics*, *50*, pp.42-49.
5. Höök P., Brito-Robinson T., Kim O., Narciso C., Goodson H., Weisel J., Alber M., Zartman J. (2017) Optical clearing and 3D fluorescence imaging of blood clots. *Biomedical Optics Express*, 8, 3671-3686.
6. Kim O.V., Litvinov RI, Chen J, Chen DZ, Weisel JW, Alber MS. (2017b) Compression-induced structural and mechanical changes of fibrin-collagen composites. *Matrix Biology*. 60, 141-156.
7. Kim O.V., Liang X., Litvinov R.I., Weisel J.W., Alber M.S., Purohit P.K. (2016) Foam-like compression behavior of fibrin networks*. Biomechanics and Modeling in Mechanobiology*, 1-16.
8. Kim O.V., Litvinov, R. Weisel J., and Alber M.S. (2014) Nonlinear structural mechanics of fibrin networks under compression, *Biomaterials*, 35, 6739-6749.
9. Kim, E., Kim, O.V., Machlus, K.R., Liu, X., Kupaev, T., Lioi, J., Wolberg, A.S., Chen, D.Z., Rosen, E.D., Xu, Z. and Alber, M., 2011. Correlation between fibrin network structure and mechanical properties: an experimental and computational analysis. *Soft Matter*, *7*(10), pp.4983-4992.
10. Wu, Z., Xu, Z., Kim, O., and Alber, M. (2014) Three-dimensional multi-scale model of deformable platelets adhesion to vessel wall in blood flow. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* 372(2021), 20130380.
11. Kim, O., Xu, Z., Rosen, E., and Alber M. (2013) Fibrin networks regulate protein transport during thrombus development. *PLoS Computational Biology*, 9(6), e1003095.
12. Xu, Z., Kim, O., Kamocka, M., Rosen, E. D. and Alber, M. (2012) Computational Models of Thrombogenesis. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine*, 4(3):237-46.
13. Brown, A.E., Litvinov, R.I., Discher, D.E., Purohit, P.K. and Weisel, J.W., 2009. Multiscale mechanics of fibrin polymer: gel stretching with protein unfolding and loss of water. *science*, *325*(5941), pp.741-744.
14. Gersh, K.C., Edmondson, K.E. and Weisel, J.W., 2010. Flow rate and fibrin fiber alignment. *Journal of Thrombosis and Haemostasis*, *8*(12), pp.2826-2828.
15. Xu S, Alber M., Xu Z. (2018) Three-phase Model of Visco-elastic Incompressible Fluid Flow and its Computational Implementation. Comm. Comput. Phys. (to appear).
16. Britton S., Kim O., Xu Z., Alber M., Weisel J. (2018) Structural and Mechanical Changes in Cohesive Fibrin Networks under Tensile Load. (in preparation).
17. Tutwiler, V., Litvinov, R.I., Lozhkin, A.P., Peshkova, A.D., Lebedeva, T., Ataullakhanov, F.I., Spiller, K.L., Cines, D.B. and Weisel, J.W., 2015. Kinetics and mechanics of clot contraction are governed by the molecular and cellular composition of the blood. *Blood*, pp.blood-2015.