Molecular Transport within Thrombi Based on In-Vivo 3D Confocal Imaging with Single Platelet Resolution

Roman S. Voronov[1], Timothy J. Stalker, Lawrence F. Brass, and Scott L. Diamond

Chemical & Biomolecular Engineering, U of Pennsylvania, Philadelphia, PA 19103-6383 USA

Lack of proper thrombus formation can result in bleeding disorders. Conversely, excessive pathogenic thrombosis and/or embolism can lead to temporary or permanent obstruction to blood flow, which results in over 1 million heart attacks or strokes each year in the US. Recently, in-vivo studies have reported that the thrombus structure is heterogeneous, consisting of a compact core of activated platelets near the blood vessel injury and a loose shell of quiescent platelets on the outer periphery of the thrombus. Motivated by the ability of intravital microscopy experiments to image the thrombus structure with a single platelet resolution, we set out to study the microenvironment within the heterogeneous thrombus structure, as well as to try and identify any possible implications that it could have on drug target design.

The approach implemented in this study is to: perform in-vivo laser injury experiments in mouse cremaster muscle arterioles, image the resulting thrombus structures using fluorescent confocal microscopy with 0.216 micron resolution and model the fluid dynamic environment experienced by the thrombus using supercomputing. Stochastic tracer tracking simulations are performed in order explore diffusive transport of different-sized coagulation factors within the thrombus, released either from the injury site or from the lumen using passive Lagrangian Scalar Tracking with a Brownian motion component. Because the Brownian contribution to the motion of molecules is largely a function of their size, molecular weights of Calcium2+ ion, ADP and Factor X were chosen as the representative low, medium and high tracer sizes, respectively. Two modes of tracer release were investigated: from the blood stream to the site of the injury and vice versa.

The tracer tracking simulations show that smaller-sized molecules (such as Ca2+ ion) penetrate or exit the thrombus in less than a second, while it takes almost 10 seconds to do the same for larger molecules (like Factor X). Similarly, the smaller tracers experience approximately an order of magnitude higher rate of collisions with the thrombus surface than do the larger ones, due to the difference in diffusive dynamics of the molecules. Unexpectedly, it was also observed that the presence of flow around the thrombus facilitates the removal of molecules from its pore space. Due to this effect, molecules of any size are not able to reach the shell of the thrombus when they were released from the injury site.

The observed differences in the microenvironment of the core and the shell regions could explain the existence of the heterogeneous thrombus structure arrangement. For example, the reason why that the shell is not compact could be due to the fact that molecules that are needed for platelet activation are released from the injury site and simply cannot reach the shell portion of the thrombus due to elimination by the surrounding blood flow. Moreover, if the observed differences between transport processes in the core and the shell could be exploited, the findings reported in this work could have implications for therapeutic strategies that target drug delivery to local areas within the thrombus structure. For example, drugs that need to penetrate the core should be made from smaller more dynamic molecules, while drugs that need to target the shell selectively (which is responsible for embolism) while at the same time leaving the core intact (thereby not causing bleeding risks) should be made from larger-sized molecules.

[1] Corresponding author email: rvoronov@seas.upenn.edu