Chemical, Pressure, and Shear Rate Gradients in Multiscale Blood Systems Biology

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In multiscale systems biology, fundamental properties such as chemical and cellular concentrations, fluid shear forces, or solid forces can vary in space and time. We report three fundamental studies highlighting chemical, pressure, and shear rate gradients:

Blood clots are rapidly assembled hemodynamic sensors: Collagen supported formation of a 20-μm thick platelet layer, which unexpectedly underwent massive platelet retraction upon flow cessation. This contraction resulted in a 5.34-fold increase in permeability due to collagen restructuring. Without stopping flow, platelet deposits (no fibrin) had a permeability of $\kappa_{platelet}$ = 5.45 x 10^{-14} cm^2 and platelet-fibrin thrombi had $\kappa_{thrombus}$ = 2.71 x 10^{-14} cm^2 for ΔP = 20.7 to 23.4 mm-Hg, the first ever measurements for clots formed under arterial flow (wall shear rate = 1130 s^{-1}). This triggered contraction was blocked by the myosin IIA inhibitor blebbistatin and by inhibitors of thromboxane (TXA_2) and ADP signaling. Also, flow cessation triggered platelet intracellular calcium mobilization, which was blocked by TXA_2/ADP inhibitors. Flow dilution of platelet autocrine signaling during intraluminal clotting balances the platelet contractile apparatus with prevailing hemodynamics, a newly defined flow sensing mechanism.

Imaging thrombin gradients in vitro and in vivo: We developed the first sensor capable of revealing inner clot thrombin gradients. An N-terminal-azido thrombin-sensitive fluorescent peptide (ThS-P) with a thrombin-releasable quencher was linked to anti-CD41 using click chemistry to generate a thrombin-sensitive platelet binding sensor (ThS-Ab). In a microfluidic assay of whole blood perfusion over collagen ± linked TF (wall shear rate = 100 s-1), ThS-Ab fluorescence increased between 90 to 450 s for 0.1 to 1 molecule-TF/um2 and co-localized with platelets near fibrin. Using a microfluidic device to control the pressure drop across a thrombus forming on a porous collagen/TF plug (521 s-1), thrombin and fibrin were detected at the thrombus-collagen interface at zero pressure drop, while 80 % less thrombin was detected at 3200 Pa in concert with fibrin polymerizing within the collagen. With anti-mouse CD41 ThS-Ab deployed in a mouse laser injury model, the highest levels of thrombin arose between 40 and 160 s nearest the injury site where fibrin co-localized and where the thrombus was most mechanically stable. ThS-Ab reveals thrombin locality, which depends on surface TF, flow, and intrathrombus pressure gradients.

Shear gradients and shear stress on vWF structure/function: In severe stenosis, vWF experiences millisecond exposures to pathological wall shear rates (γ_w). We deployed microfluidic devices for single-pass perfusion of whole blood or platelet-free-plasma (PFP) over fibrillar type 1 collagen (< 50 msec transit time) at pathological γ_w or spatial wall shear rate gradient (grad γ_w). Using fluorescent anti-vWF, long thick vWF fibers (>20 μm) bound to collagen were visualized at constant γ_w > 30,000 s^{-1} during perfusion of PFP, a process enhanced by EDTA. Rapid acceleration or deceleration of EDTA-PFP at grad γ_w = ± 5.5 x 10^5 to 4.3 x 10^7 s^{-1}/cm did not promote vWF deposition when γ_w < 30,000. At 19,400 s^{-1}, EDTA-blood perfusion resulted in rolling vWF-platelet nets, while blood perfusion (normal Ca^{2+}) generated large vWF/platelet deposits that repeatedly embolized and were blocked by anti-GP1b or the αIIbβ3 inhibitor GR144053 and did not require shear gradients. Blood perfusion at venous shear rate (200 s^{-1}) produced a stable platelet deposit that was a substrate for massive but unstable vWF-platelet aggregates when flow was increased to 7800 s^{-1}. Supported by collagen and enhanced by platelet GP1b and αIIbβ3, vWF fiber formation occurred during acute exposures to pathological γ_w but did not require wall shear rate gradients.