528b Pharmacologically-Induced Changes in Neutrophil Membrane Mechanics Regulate the Psgl-1/P-Selectin Adhesion Lifetime

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Visualization of flowing neutrophils colliding with adherent 1-micron diameter beads presenting P-selectin allowed the simultaneous measurement of collision efficiency (ε), membrane tethering fraction (f), membrane tether growth dynamics, and PSGL-1/P-selectin binding lifetime. For 1391 collisions analyzed over venous wall shear rates from 25 to 200 sec^{-1}, ε decreased from 0.17 to 0.004, while f increased from 0.15 to 0.70, and the average projected membrane tether length, L^m_{tether}, increased from 0.35 μm to ~2.0 μm over this shear range. At all shear rates tested, adhesive collisions lacking membrane tethers had average bond lifetimes less than those observed for collisions with tethers. For adhesive collisions that failed to form membrane tethers, the regressed Bell parameters (consistent with single bond Monte Carlo simulation) were: zero-stress off-rate, $k_{off}(0) = 0.56\ sec^{-1}$ and reactive compliance, r = 0.10 nm, similar to published AFM measurements. For all adhesion events (± tethers), the bond lifetime distributions were more similar to those obtained by rolling assay and best simulated by Monte Carlo with the above Bell parameters and an average of 1.48 bonds [n = 1 bond (67%), n = 2 (22%), n = 3-5 (11%)]. For collision at 100 sec^{-1}, pretreatment of neutrophils with actin depolymerizing agents, latrunculin or cytochalasin D, had no effect on ε, but increased L^m_{tether} by 1.74- or 2.65-fold and prolonged the average tether lifetime by 1.41- or 1.65-fold, respectively. Jasplakinolide, an actin polymerizing agent known to cause blebbing, yielded results similar to the depolymerizing agents. Conversely, cholesterol-depletion with methyl-β-cyclodextrin or formaldehyde fixation had no effect on ε, but reduced L^m_{tether} by 66 or 97% and reduced the average tether lifetime by 30 or 42%, respectively. The neutrophil-bead collision assay combines advantages of AFM (small contact zone), aggregometry (discrete interactions), micropipette manipulation (tether visualization), and rolling assays (physiologic flow loading). Membrane tether growth can be enhanced or reduced pharmacologically with consequent effects on PSGL-1/P-selectin lifetimes.