Supplemental Material

Non-adhesive collisions between cells in the free stream and substrate bound cells do not affect primary capture frequency

Upon increasing the inlet cell concentration (Fig.7), we observed higher rolling and adherent cell densities. Besides augmenting the frequency of cell-substrate collisions, increasing cell densities may also affect two other features: i) It may increase tethering flux due to enhanced secondary tethering. ii) Non-adhesive collisions between cells in suspension and substrate-bound cells may alter primary capture frequency. The rolling and adherent cell density may thus be changed. With regard to the latter point, it has been shown that introduction of red blood cells at 30% hematocrit can dramatically alter the cell rolling velocity and density (Munn, L.L., et al. *Biophys J.* **71**:466-478, 1996).

We examined if under our experimental conditions, which consisted of dilute cell suspensions, non-adhesive interactions between the cells may alter the primary capture frequency, θ_{fr} (Fig.1S). To assess this, 'non-adhesive' neutrophils were prepared by stimulating normal neutrophils with 1μM fMLP followed by fixation with 2% glutaraldehyde[1]. Tethering via these 'non-adhesive' neutrophils was low at 10% of the normal cells (Fig.1S). Experiments with a combination of non-adhesive and normal neutrophils were then performed. In these runs, we observed that the addition of 0.13×10^6 non-adhesive cells/ml to 0.07×10^6 normal neutrophils/ml did not significantly alter either the rolling velocity or the number of rolling/adherent cells in

[1] **Method for preparation of "non-adhesive" neutrophils**: Isolated neutrophils were stimulated with 1 μM formyl peptide (fMLP) for 10 min to shed the L-selectin (Taylor, A.D., et al., *Biophys J.*, **71**:3488, 1996) and to redistribute and/or shed the selectin ligands on neutrophils (Davenpeck, K.L., et al. *J Immunol.* **165**:2764, 2000). We have also shown that stimulation of this nature reduces cell adhesion via the β_2-integrin subunits, LFA-1 and Mac-1 to baseline levels (Neelamegham, S., et al., *Blood.* **92**:1626, 1998). These deactivated cells were then washed by rapid centrifugation, and resuspended in HEPES buffer containing 2% glutaraldehyde. After fixation in glutaraldehyde for 30 min at room temperature, the cells were washed and then resuspended in regular HEPES buffer. Cells were stored at 4°C until experimentation. Cell count was performed using a coulter counter (Model ZM, Coulter Electronics, Beds, England) to verify the concentration of cells used in the flow chamber runs.

comparison to runs with $0.07x10^6$ normal cells/ml alone (in the absence of non-adhesive cells). Thus, the pattern of neutrophil rolling and firm-arrest in experiments with dilute cell suspensions is not affected by non-adhesive interactions between cells in the free-stream and substrate bound cells. Overall, while other studies have noted that erythrocytes in human blood may alter leukocyte recruitment rates (Munn et al., 1996), our results suggest that this is not the case in typical flow chamber experiments with dilute cell suspensions.

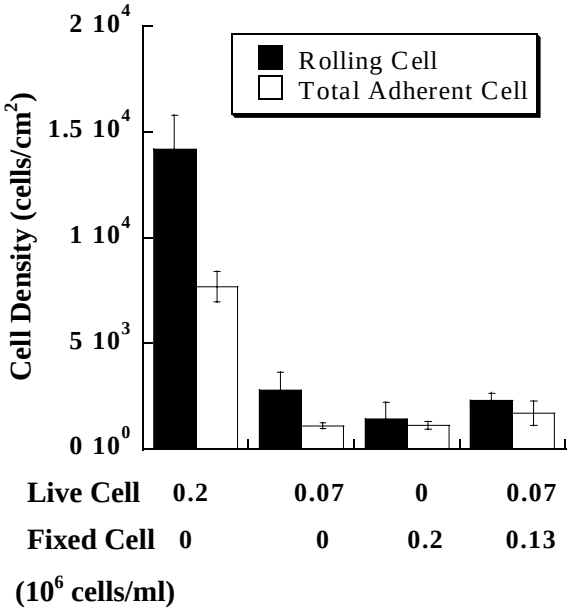

Figure 1S: Effect of cells in the free stream on rolling and adherent cell density Neutrophils were stimulated with 1μM formyl peptide for 10 min to make the cells non-adhesive, fixed in 2% glutaraldehyde and then washed by rapid centrifugation. These non-adhesive or 'fixed' cells were then introduced into a flow chamber containing an E/I cell substrate at τ_ω=2 dyn/cm^2, either in the absence or presence of other unactivated 'live' neutrophils. Rolling and adherent cell density at 10 min is reported. Fixed cells themselves did not bind to E/I cells. Addition of fixed cells along with live cells also did not significantly affect the number of live cells that rolled or adhered. Error bars are mean$\pm$SEM for N$\geq$3.