Electronic Supplementary Material (ESI) for Integrative Biology

Selectin-mediated Adhesion in Shear Flow Using Micropatterned Substrates: Multiple-Bond Interactions Govern the Critical Length for Cell Binding

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Supplementary Information

Table S1. Comparison between a typical Glycotech flow chamber with a microfludic flow assay system.

	Glycotech flow chamber	Microfludics flow assay
Typical dimensions* (H x W x L)	200 µm x 1 cm x 3 cm	20 µm x 1500 µm x 2 cm
Working volume	mL	µL
Vacuum pump requied	Yes	No
Reusable	Yes	Yes
Mode of fluid flow	Syringe pump	Syringe pump
Cell streaming velocity	Varied depending on the location	Uniform with minor deviation
Cell flux calculation	Difficult[#]	Easy

* The dimensions of a microfluidic device can be easily manipulated in lab setting, while the Glycotech flow chamber dimensions are predetermined.

[#] Cell flux at the bottom plate is a function of distance from the cell source entrance, bulk cell concentration and flow rate.

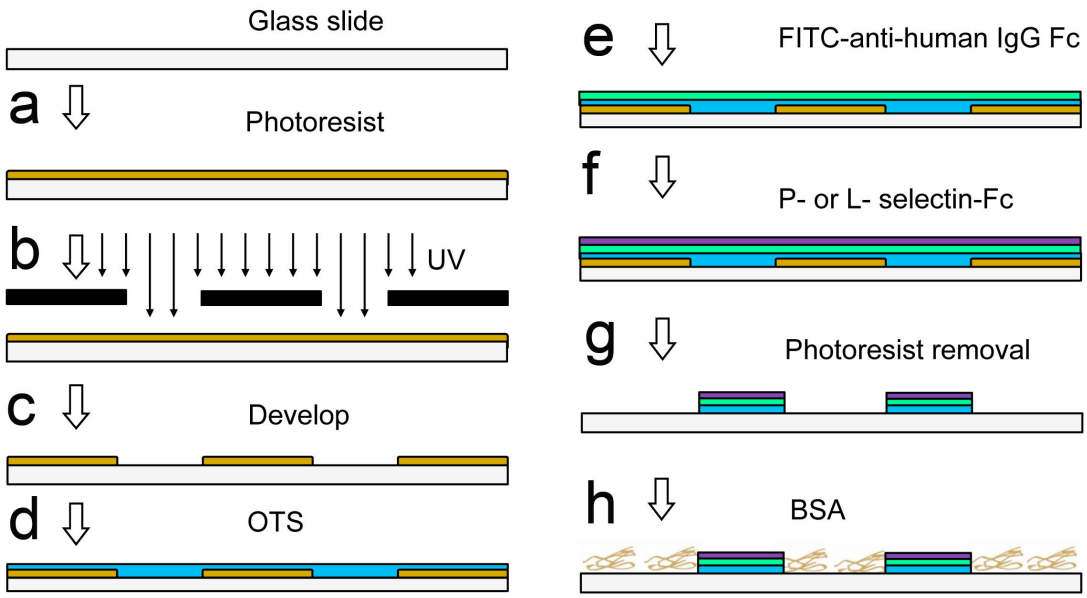

Figure S1. Flow schematics of micropatterning of selectins. (a) Photoresist was spin-coated on a precleaned microscope glass slide and (b) exposed to UV light through a chrome mask. (c) The irradiated micro-regions of photoresist were removed by developer. (d) The patterned glass slide was then immersed in octadecyltrichlorosilane (OTS) solution. (e) FITC conjugated goat anti-human IgG, Fc fragment specific antibody was incubated on the slide and then with (f) P- or L- selectins. Lastly, (g) the photoresist was completely removed by photoresist remover. (h) The slide was then incubated with 1% BSA solution for an hour for blocking nonspecific bindings prior to microfluidic flow assay.

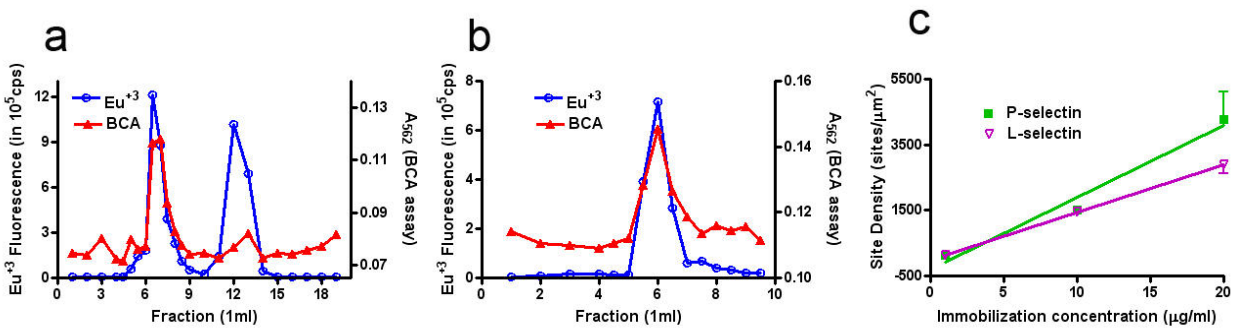

Figure S2. **P- and L- selectin labeling and purification.** (a) L-seletin and (b) P-selectin were incubated with excess europium chealates and purified via Superdex G50 packed column. Different fractions were characterized by time-resolved fluorescence intensity measurement (indentifying europium ion fractions) and BCA assay measurement (identifying protein fractions). (c) The purified europium-conjugated selectins were used for site density measurements. Data represent triplicate experiments with mean $\pm$ SEM.

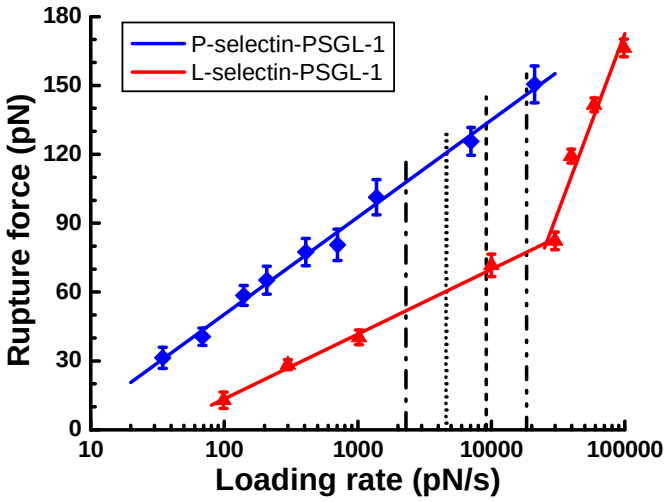

Figure S3. Estimation of the bond strength between P-/L-selectin binding to PSGL-1. The experimental data of rupture force vs. loading rate was adopted from Evan et al. (1, 2). Data (symbols) are connected by fitting curves (color solid lines) to estimate the tensile strength of a P-/L-selectin-PSGL-1 bond on a tethering HL-60 cell under flow. The loading rates of hydrodynamic force at 0.25, 0.5, 1 and 2 dyn/cm^2 were calculated by Equation 2 as marked by black broken lines. The corresponding bond strengths of P-/L-selectin-PSGL-1 bond were obtained at the intersection points between the blue/red fitting curves and black broken lines.

Reference

1. Evans, E., A. Leung, D. Hammer, and S. Simon. 2001. Chemically distinct transition states govern rapid dissociation of single L-selectin bonds under force. Proc Natl Acad Sci U S A 98:3784-3789.
2. Evans, E., A. Leung, V. Heinrich, and C. Zhu. 2004. Mechanical switching and coupling between two dissociation pathways in a P-selectin adhesion bond. Proc Natl Acad Sci U S A 101:11281-11286.

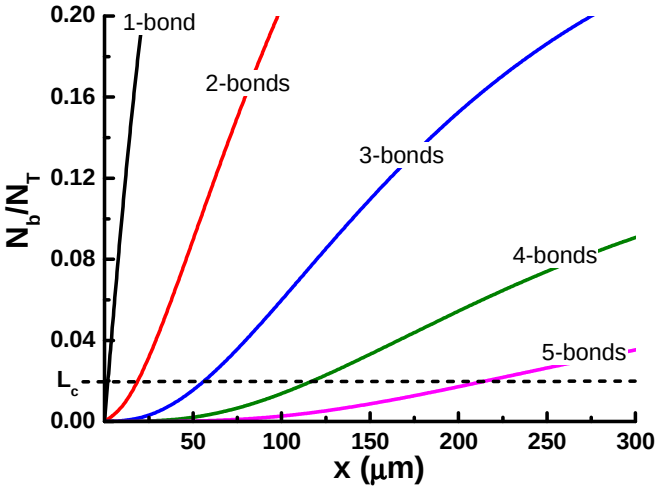

Figure S4. Multiple-bond interaction governs the critical patch length (L_c) for initiation of cell tethering. The cell tethering rate (N_b/N_T) is calculated at different minimum number of bonds under a constant shear level. Our model predicts that a longer patch is needed to initiate cell tethering at a larger number of bonds. The critical patch length (L_c) marked by a dashed line is selected as the distance required for mediating 2% of cell tethering rate. For instance, L_c=20 μm when cell tethering is mediated by 2 bonds, whereas a significantly longer patch (~200 μm) is required for that mediated by 5 bonds.