High-Throughput, Physiological Assays of Leukocyte Adhesion and Rolling

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ABSTRACT

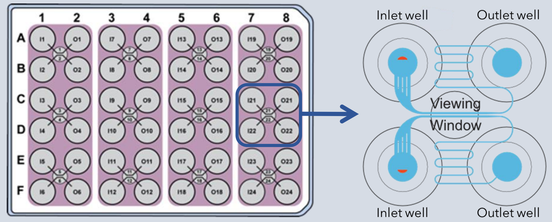
Introduction: The leukocyte adhesion cascade is critical for inducing and resolving inflammation during the immune response to pathogens and tissue injury. Although assays exist to examine various steps of the leukocyte adhesion cascade, the initial step of adhesion is typically assessed in static plates, limiting physiological relevance. Additionally, leukocyte rolling must be assessed under shear stress; however, homemade flow cells and slide-based flow chambers are tedious and low-throughput. Here we present data demonstrating how the leukocyte adhesion cascade steps of adhesion and rolling can be quantified in straightforward, high-throughput, and physiologically relevant assays using a BioFlux shear flow system. **Methods:** Jurkat cell adhesion and rolling in response to cations and antiadhesion treatments was assessed. In addition, changes in cell adhesion strength in response to varying concentrations of fibronectin under increasing shear stress was quantified. Finally, rolling speed of primary lymphocytes on PNAd was assessed. **Results:** No adhesion or rolling was observed in the absence of divalent cations. 1 mM magnesium (Mg^{2+}) elicited cell adherence without rolling, 1 mM calcium (Ca^{2+}) elicited slow rolling, and Ca^{2+} and Mg^{2+} together resulted in slow rolling. Adhesion increased in response to increasing concentrations of fibronectin, and adhesion strength was not altered by shear rates <60 dyn/cm^2. Lymphocyte rolling velocitry was increased with increasing shear stress. **Conclusion:** Physiologically relevant leukocyte adhesion, adhesion strength, and rolling velocity can be imaged and quantified in high-throughput using a BioFlux shear flow system.

INTRODUCTION

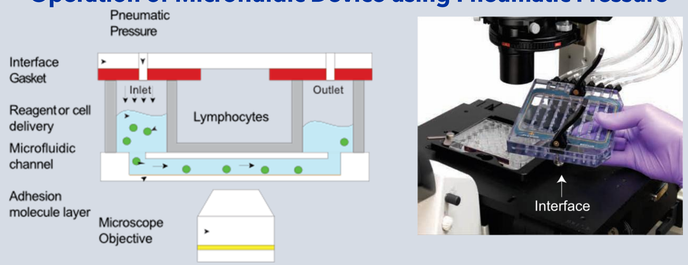
- Leukocyte adhesion is involved in a normal immune response as well as the pathogenesis and progression of diseases, such as atherosclerosis.
- Static assays of leukocyte adhesion often do not translate well to in vivo systems. Assays of leukocyte rolling, using traditional flow cells and slide-based shear flow systems, are overly complicated and have low-throughput.
- A straightforward, high-throughput shear flow system capable of assaying multiple steps of the leukocyte adhesion cascade would increase both the quantity and quality of data from immunology investigations.

METHODS

Microfluidic Plate for Adhesion Cascade Assays



Operation of Microfluidic Device using Pneumatic Pressure



Experimental Design

- The outlet wells of a BioFlux plate were used to coat the microfluidic channels with either vascular cell adhesion molecule 1 (VCAM-1), fibronectin, or peripheral node addressins (PNAd)
- Outlet wells were used to perform washes and blocking
- Leukocytes suspended in the condition of interest were introduced to the channels for the indicated times and shear flow rates
- All data were captured using an inverted microscope

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- Image and quantify physiological leukocyte adhesion and rolling
- Conduct multiple assays with one system
- Examine various conditions/replicates in parallel

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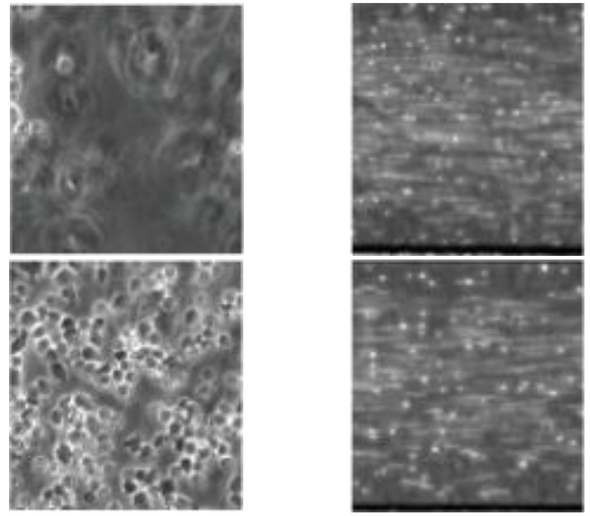
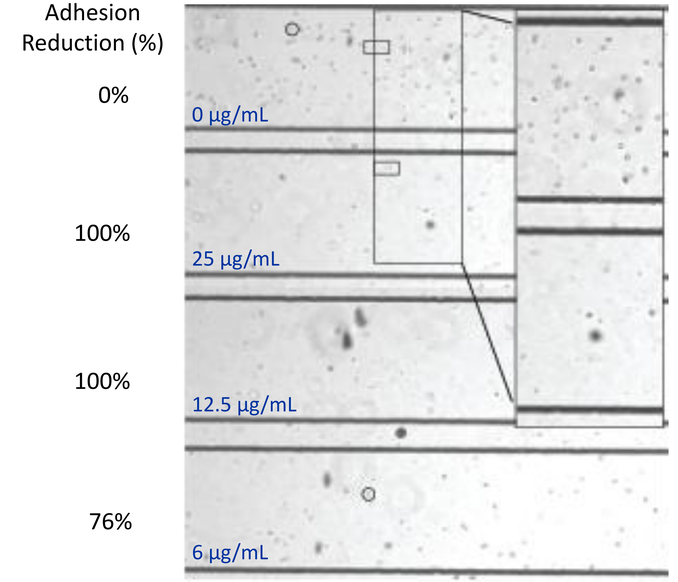
RESULTS

Adhesion Response to Divalent Cations



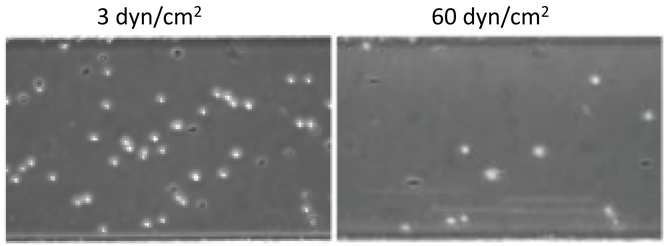
Jurkat cells were flowed into VCAM-1 coated microfluidic channels. Shear stress for all divalent cation experiments was 2 dyn/cm^2. No rolling or adhesion was observed in the absence of divalent cations. 1 mM Ca^{2+} resulted in slow rolling, 1 mM Mg resulted in adherence without rolling, and 1 mM Ca^{2+} + 1 mM Mg^{2+} resulted in slow rolling.

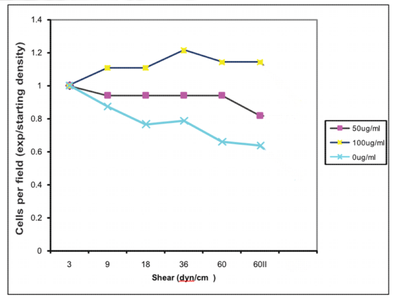
Inhibition of VCAM-1



Microfluidic channels were coated with 10 µg/mL VCAM-1 and blocked with 1% BSA. Specific binding was blocked by adding various concentrations of anti-VCAM-1 (R&D Systems) to the channels. Jurkat cells were flowed at 1 dyn/cm^2 for 10 minutes. Cells per microscopic field were counted using 3 fields per channel across the viewing window. Adhesion decreased with increasing anti-VCAM-1.

Adhesion Strength





Jurkat cells were flowed into fibronectin coated microfluidic channels. Shear flow rate was started at 3 dyn/cm^2 and increased every 3 minutes up to 60 dyn/cm^2. All data were captured under shear flow. In channels coated with 100 µg/mL fibronectin, adherence increased up to 36 dyn/cm^2 and then plateaued. In channels without fibronectin, cell detachment increased with increasing shear rate.

Rolling Velocity

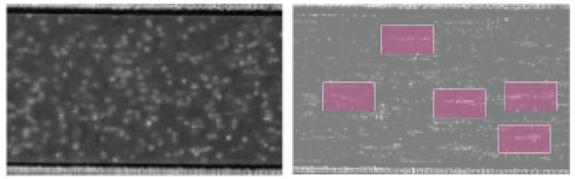


Table 1.

PNAd Dilution	Shear Stress (dyn/cm^2)	Avg. Rolling Velocity (µm/sec)
1:20	3	113
1:20	4	115

Lymphocytes were flowed into channels coated with the lymphocyte homing receptor ligands, PNAd (right). Time-lapse image stacks were collected under various shear stresses, superimposed, and spatially filtered to enhance the measurement of cell tracks (middle - shaded boxes). Table 1 shows the results of cell tracks measurements at two shear stress values.

CONCLUSION

- Jurkat slow rolling under low shear stress is calcium-dependent
- Anti-VCAM-1 inhibits Jurkat adhesion to VCAM-1 under low shear stress
- Jurkat adhesion strength is reduced with increasing shear stress and is dependent on fibronectin concentration
- Jurkat adhesion strength to fibronectin is not affected by shear stresses lower than 60 dyn/cm^2
- Lymphocyte rolling velocity is slightly increased by increasing shear stress