LB448

# A High-Throughput Automated Microfluidic Platform for Live Cell Rolling and Dynamic Adhesion Assays

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## Abstract

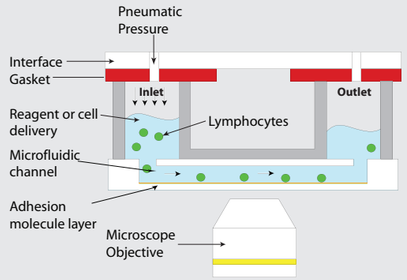
A large number of processes underlying immune system biology are dependent on leukocyte adhesion and neutrophil rolling phenomena. Laminar flow chambers are the standard tool in vitro for studying cellular adhesion under shear stress conditions, but are of limited use for assays with high numbers of variables, eg. chemical library screens, because of low throughput, poor response time, and long setup procedures. Here we present development of a disposable microfluidic platform which improves upon throughput, experimental control, and setup time for cell adhesion and cell rolling assays. Rolling adhesion assays were performed in the presence or absence of divalent cations on this platform on either VCAM-1 or PNAd. These data were compared to established results presented in the literature. Consistent with the established data (Chen, et al., 2004), no adhesion or rolling was observed in the absence of divalent ions. Buffer conditions with 1mM magnesium (Mg) resulted in cell adherence with no rolling; addition of 1mM calcium (Ca) without Mg resulted in slow rolling while including both Ca and Mg resulted in a similar slow rolling. Jurkat cell adhesion strength to fibronectin under shear flow and adhesion dose response was also successfully assessed using the microfluidic platform. Principal advantages demonstrated with the BioFlux 200TM system were parallelization of assays, real-time imaging, minimized reagent consumption, precise control over shear stress, integrated image acquisition, and automated cell tracking analysis.

## Experimental Design

Microfluidic channels were coated with either VCAM, fibronectin, or PNAd from the outlet well. If needed, washes and blocking were also performed from outlet wells.

Jurkat cells suspended in condition of interest were introduced to the channels at a given shear for a set amount of time from the inlet wells.

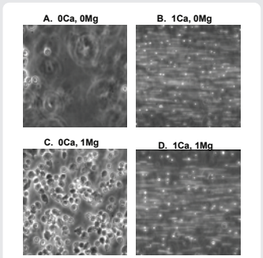
Data were captured using an inverted microscope.




## Jurkat Cell Adhesion: Divalent Cations

The purpose of this experiment was to demonstrate the effects of divalent ions Mg and Ca on cellular adhesion of Jurkat cells to a VCAM-1 coated glass substrate.

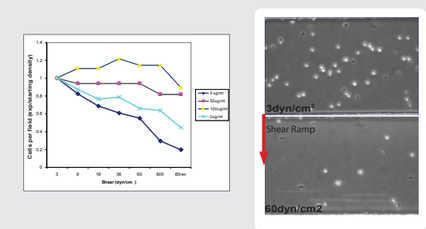
Jurkat cells were prepared as described and applied to VCAM-1-coated channels in the presence of the indicated concentrations of Ca and Mg (in mM). Shear stress in all panels was 2 dyne/cm2. In agreement with the literature, (Chen, et al, 2004; J. Biol. Chem. 279:55556-55561.) in the absence of divalent ions no adhesion or rolling was observed (A). Adding 1mM Mg resulted in cell adherence with no rolling (C). Adding 1mM Ca without Mg resulted in slow rolling (B) while including both Ca and Mg resulted in a similar slow rolling (D).



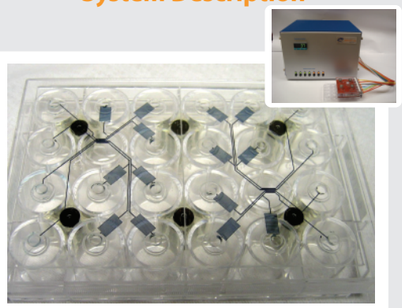

## Adhesion Strength of Lymphocytes on Human Fibronectin

This experiment was designed to demonstrate the effects of a controlled shear ramp on Jurkat cell adhesion strength to fibronectin.

Channels were coated with human fibronectin and briefly washed with HBSS. Jurkat cells were introduced to the channels at 3 dyn/cm2 for 10 minutes. A shear ramp was initiated beginning at 3 dyn/cm2, increasing every 3 minutes until shear reached it maximum. Data was captured under flow to distinguish between adherent and non-adherent cells as well as to maintain continuous application of force. On channels with 100µg/ml fibronectin, cells continued to adhere until 36 dyn/cm2 was reached, but no significant decrease in attachment was noted indicating a very strong interaction. At the lowest concentration of fibronectin, cells detached a continuous manner as shear increased, indicating the adhesion was not strong under these conditions. At all concen-




## System Description



The BioFlux™200 system is:

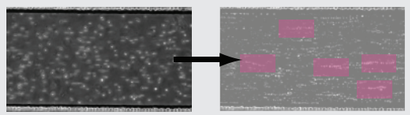
A BioPlate (above) multi-well plate with up to 24 shear controlled microfluidic channels, a.k.a.micro-sized flow cells, coupled to the wells. All reagents and cells are loaded in the wells to be introduced into the microfluidic channels.

The BioFlux controller (inset) delivers shear flow from 0.5-20 dyn/cm². It is controlled by the BioFlux software, which also supports imaging and image analysis.

## Lymphocyte Rolling on PNAd-Coated Substrate

The objective of this experiment was to demonstrate the measurement of lymphocyte rolling in the BioFlux device. Cells and adhesion molecules were kindly provided by the lab of Steven Rosen at UCSF.

Lymphocytes were introduced into BioPlates coated with a well-characterized cell adhesion factor, PNAd (addressin). Time-lapse image stacks were collected under various values for shear stress. Time-lapse image stacks were superimposed and spatially filtered to enhance the measurement of cell tracks (shaded, below). Cellular tracks were measured at two similar values of shear stress and the averaged results are presented in the Table, below.



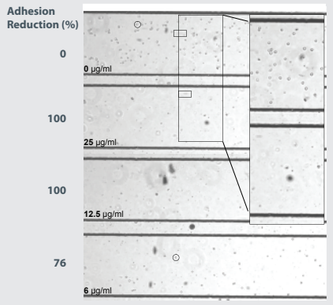
Time-Laspe Stack

| | | Average Rolling Velocity |
|---|---|---|
| PNAd 1:20 dilution | 3 dyn/cm² | 113 µm/sec |
| PNAd 1:20 dilution | 4 dyn/cm² | 115 µm/sec |

## Adhesion Inhibition: anti-VCAM

In order to evaluate inhibition of Jurkat cell adhesion in the BioFlux system, we used a primary coating of VCAM in the channels, followed by a secondary coating of anti-VCAM.

Microfluidic channels were coated with 10µg/ml VCAM. Channels were blocked with 1%BSA; anti-VCAM (R&D systems)was added to the channels at various concentrations. Jurkat cells suspended in 1% BSA were introduced to the channels at 1dyn/cm2 for 10 minutes. Channels were washed to remove extra cells. Cells per microscopic field were counted; 3 fields were captured per channel covering the entire length of the viewing window.




## Conclusions

**A variety of standard assays to study cell adhesion were successfully adapted for use with and performed in the BioFlux 200 system.**

Jurkat cells were found to roll and adhere to VCAM and PNAd in the presence of divalent cations within microfluidic channels of the BioFlux plate.

In the absence of Mg and Ca, cells did not roll on VCAM; rolling only occurred with Ca or Ca plus Mg.

Adhesion to VCAM can be completely inhibited by anti-VCAM antibodies.

Adhesion strength of Jurkat cells to fibronectin depends on the concentration.