

Cellular Rolling Velocity

Inflammation model using Jurkat cells and VCAM-1

Introduction

Visualization of cellular processes in real-time provides invaluable insight into cell biology. Cell adhesion through protein-protein or protein-carbohydrate interactions plays an important role in directing cell migration, gene expression, cell growth, morphological changes during development, and programmed cell death.

VCAM-1 is a cell adhesion molecule expressed on the endothelial surface during inflammation. VLA-4 is a $\beta 1$ integrin expressed on the surface of lymphocytes, monocytes and eosinophils. VLA-4 has been shown to interact with VCAM-1 in the context of lymphocyte homing to inflammation; lymphocyte rolling and adhesion to VCAM-1 has been previously demonstrated (Alon et al, 1995) using traditional and flow-based methods. The BioFlux System (Figure 1) is an easy to use, automated platform for investigation of biological processes under shear flow. Here, we present Jurkat cell rolling using live cell imaging and the BioFlux 200 system.

Methods

A 48-well BioFlux Plate was used, which contains 24 individual flow cell experiments (Figure 2). Channels were coated with recombinant human VCAM-1 (rhVCAM-1)(R&D Systems, Minneapolis, MN) at a concentration of 15 μ g/ml. Non-specific interactions were blocked using 0.5% BSA in HBSS. Jurkat cells were introduced into microfluidic channels at a shear of 2 dyn/cm^2 . Once cells were visible within the viewing window, shear was reduced to 1 dyn/cm^2 . Movies of cell rolling were captured with a Go-3 Camera (QImaging, Surrey, BC) with an exposure time of 100ms at 500ms intervals.

Results

We exploited the interaction between VLA-4 on Jurkat cells and recombinant VCAM-1, a cell adhesion molecule to model cell rolling and adhesion assays in the BioFlux system. Jurkat cells rolled on VCAM-1 at a shear of 1 dyn/cm^2 and ultimately attached to the substrate. After firm attachment, cells could be removed from the surface between shears of 7 and 30 dyn/cm^2 .

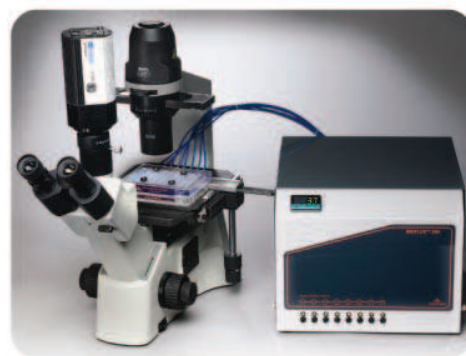


Figure 1: The BioFlux 200 System for live cell assays under controlled shear flow.

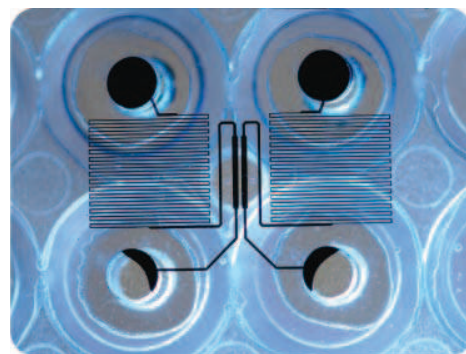


Figure 2: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

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APPLICATION NOTE

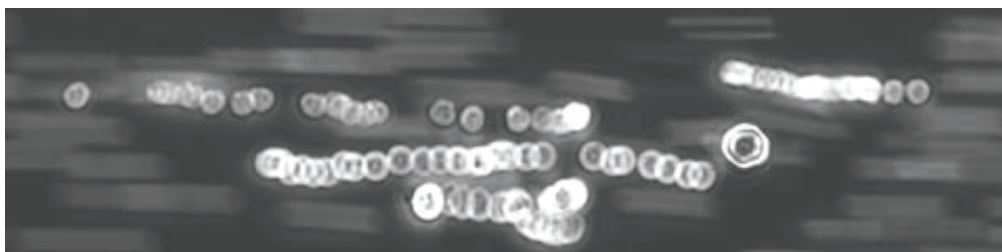


Figure 3: Jurkat cells rolling on VCAM-1. A stack of thirty consecutive images were captured 0.5 seconds apart (100 ms exposure).



Figure 4: Cell rolling from tethered state. Cells were introduced into channels for rolling assay. Cells were relieved from shear for 5 minutes and then shear was ramped up from 2 dyn/cm² to 36 dyn/cm². Cell tethers are indicated (black arrow), frame number is shown in white and encompasses shear from ~7 dyn/cm² to ~28 dyn/cm².

Cell tethers during rolling

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Appendix I: Specifications

BioFlux Plates

Available configurations:	24-well Plate (8 independent experimental channels, two inputs per channel) 48-well Plate (24 independent experimental channels, one input per channel)
Channel cross section:	350µm wide X 70µm tall
Channel materials:	polystyrene (well plate), PDMS (channel sides and roof), 180µm cover slip glass (channel bottoms and imaging path)
Shear Flow:	0.5 – 20 dyne/cm ²
Uninterrupted Run Time:	Up to 24hrs at 1 dyn/cm ² (wells can be refilled at any time)

BioFlux Controller

Dimensions:	12" wide, 13" deep, 9" tall (30cm X 33cm X 23cm)
Power Supply:	5A 12V DC (included)
Connections:	USB cable (included)

BioFlux Software and PC Requirements

Operating System:	Windows 2000, XP
Hardware Requirements:	CD drive, USB 2.0 connection (for Controller), USB or FireWire connection (for camera)



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