Cell adhesion and dose response to an adhesion inhibitor using the BioFlux™ 200 system

Application Note

Background

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-1 is a β1 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 methods. The BioFlux system is an easy to use, automated platform for investigation of biological processes under shear flow. Here, we present Jurkat cell adhesion and inhibition of adhesion using live cell imaging and the BioFlux 200 system.

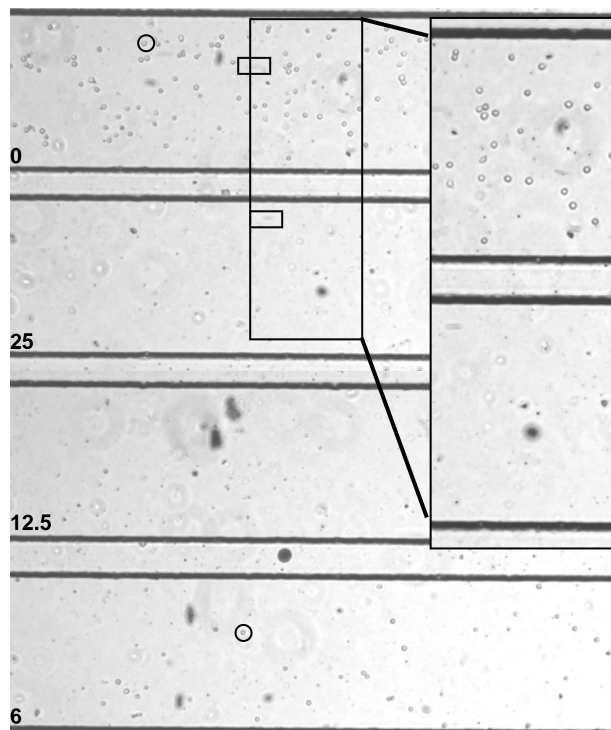
Experimental Method

Adhesion and Dose Response to Inhibitor

Channels were coated with rhVCAM-1 at 10ug/ml. Anti-VCAM-1 (R&D systems) was added at either 0, 6, 12.5 or 25 µg/ml concentration in 1% BSA in PBS and incubated for an hour at room temperature. Cells were added at a concentration of 0.5 x 10^6 cells per ml at a shear of 2 dyn/cm^2. For adhesion, shear was reduced to 1 dyn/cm^2 for a duration of 10 minutes. Fresh buffer was added to the inlet wells and perfused for an additional 10 minutes. Channels were imaged under flow to distinguish between adherent cells and any residual cells moving through the channel . Cells were counted post-imaging.

Results

Treatment	Cells/channel	Reduction (%)
No antibody	228	0
25 µg/ml	0	100
12.5 µg/ml	0	100
6µg/ml	55	76



Dynamic cell adhesion assay. VCAM-1 coated BioFlux™ channels were treated with an anti-VCAM-1 antibody at either 0, 6, 12.5, or 25 µg/ml. Cell adhesion was assessed under flow. Stationary cells (ex/ black circles) were counted and were easy to distinguish from non-adherent cells (ex/ black box). At 6µg/ml, attachment was reduced by 76% compared to untreated; at 12.5 and 25 µg/ml adhesion was completely blocked.

Summary

We exploited the interaction between VLA-4 on Jurkat cells and recombinant VCAM-1, a cell adhesion molecule to model cell adhesion assays in the BioFlux system. Cell adhesion and adhesion inhibition could be quantitated under continuous flow, providing major work flow advantages over traditional adhesion assays.

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: 400um wide X 100um tall

Channel materials: polystyrene (well plate), PDMS (channel sides and roof), 180um cover slip glass (channel bottoms and imaging path)

Shear Flow: 0.5 – 60 dyne/cm^2

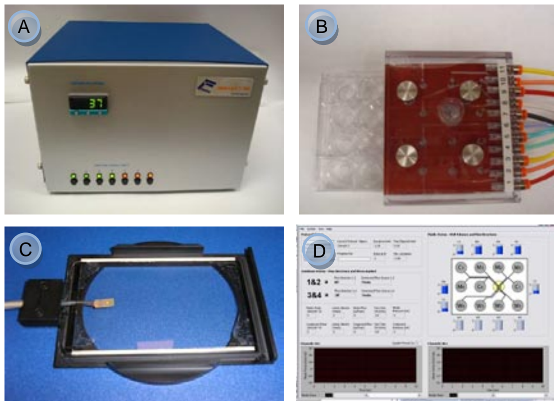
Uninterrupted Run Time: Up to 40hrs at 2dyn/ cm^2 (wells can be refilled at any time)

BioFlux™ Controller

Dimensions: 12" wide, 12" deep, 9" tall
Power Supply: 100-240 V auto-detecting (included)
Connections: USB cable (included)

BioFlux™ Software and PC Requirements

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



Bio Flux System components: A) BioFlux Controller – contains a compressor and electropneumatic regulators to control pressure output. B) Interface – serves as a pressure distribution manifold to the BioFlux Plates. C) Heating Stage – transparent indium tin oxide heating stage to control temperature during experiments. D) Software – automates many of the common, repetitive tasks needed during setup and experiment.