FLUXION

APPLICATION NOTE

Cellular Adhesion

Adhesion of colon carcinoma cells and leukocytes to endothelial cells

Introduction

Endothelial cell adhesion is the first step in a number of processes in human health and disease. In normal functioning of the immune system, white blood cells are recruited through the vascular endothelium to the underlying tissue to perform tasks related to inflammation and injury. In metastatic disease tumor cells can invade tissues using similar mechanisms of transmigration as immune cells. One of the first steps in either process is cell adhesion to the endothelial cells lining the blood vessels.

Using the Bioflux 200 system (Figure 1), a multiplexed, microscale, shear flow system optimized for tight shear control and capture of microscopy data, endothelial cell monolayers were grown to full confluence and activated with IL-1β. Leukocyte and colon carcinoma cells were introduced onto the endothelial cell monolayers under flow and adhesion data were captured using microscopy.

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

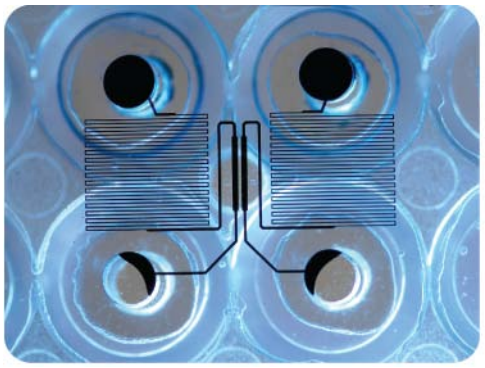

Figure 2: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

Methods

Bioflux channels (Figure 2) were pre-coated with 0.1% gelatin for 30 minutes at 37°C to foster the growth of HUVEC cells. Following the incubation with gelatin, the outlet wells were trypsinized to remove gelatin coating the well. Wells were washed with PBS to remove trypsin. Cells were added to outlet wells at a concentration of 2 x 10^6 cells/ml. Immediately, cells were pulsed into the viewing window at 2dyn/cm^2. Density was intended to be relatively high (at 50% or higher) in order to grow a confluent monolayer within 24 hours. Cells were allowed to attach to the gelatin for 2 hours at 37°C at 5% CO2. Cells were grown under gravity flow by removing excess liquid from the outlet wells and adding the maximum amount of media to the inlet wells. Cells were used after 16-20hours of incubation.

Cells were activated with IL-1β for 4.5 hours at 37°C in normal growth media. Cells were exposed to 3 dyn/cm^2 shear flow in CO_2-independant media supplemented with 0.1% BSA (test media) at 37°C before addition of adherent cells. Either Jurkat cells or HT29 cells were added to channels at a density of 1 x 10^6 cells/ml in test media. Shear was adjusted during the experiment from 3 to 1 dyn/cm^2 using the BioFlux 200 controller.

Results

Jurkat cells were perfused under 3 dyn/cm^2 and 1 dyn/cm^2 shear flow over the HUVEC monolayers. At 3dyn/cm^2, no rolling or adhesion was noted. However, when the shear was reduced for 10 minutes rolling and adhesion occurred at very high rates (Table 1). On the activated monolayers, there was almost double the adhesion when compared to resting monolayers (Table 1 and Figure 3). Under all conditions, HT29 cells exhibited slow rolling, tethering, and pausing, but very little frank adhesion (Table 1 and Figure 3).

	Jurkat	HT29
Resting	48.5	11
Activated	85.5	4

Average cells per field for 2 fields using 4Xobjective

Table 1: Average adhesion per microscopic field on HUVEC monolayers. Data represents average number of adhered cells per field of view.

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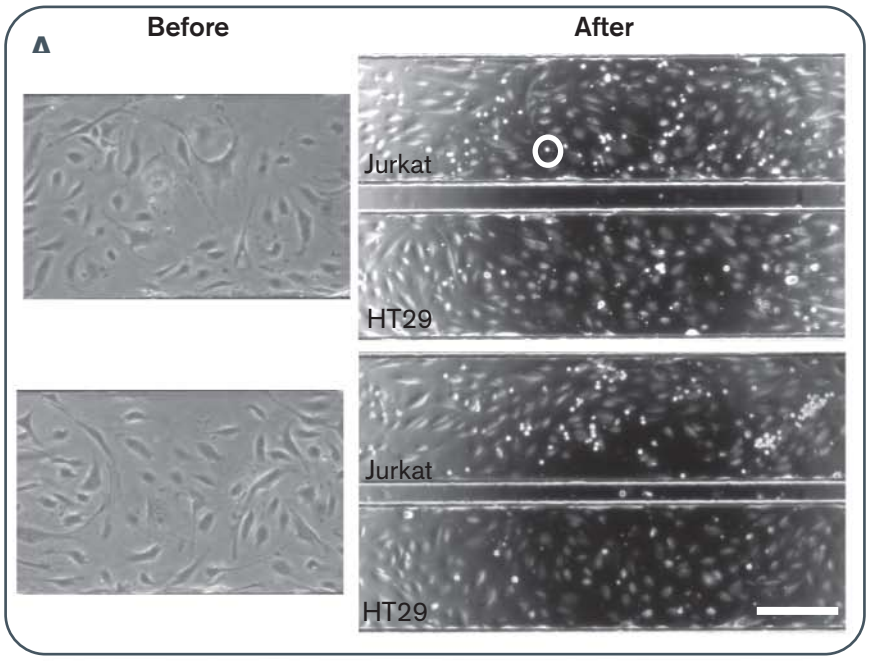

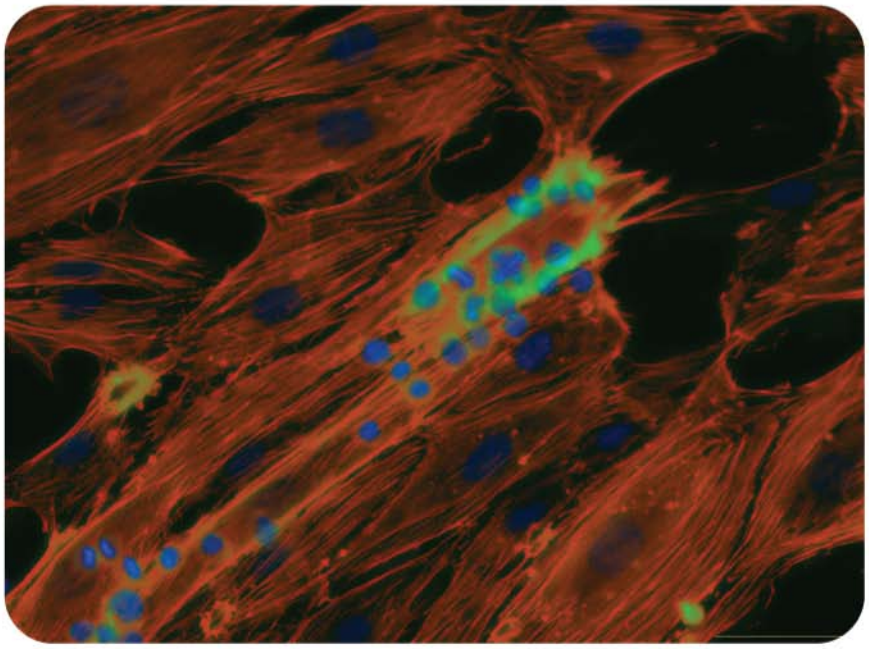

Figure 3: Cell adhesion on HUVEC monolayers.
(A) Representative monolayers before (scale bar is 100 microns) and after shear flows were applied. In the after monolayers, adherent cells appear as phase bright circles, an example is outlined with a white circle (scale bar=200 microns). Phase optics were adjusted to emphasize adherent cells.
(B) Jurkat cells attached to an activated HUVEC cell. Cells were stained for F-actin and DNA using fluorescently labeled phalloidin (red, focused on HUVEC cells, and green, focused on Jurkats) and Hoescht 33342 (blue) respectively (Scale bar=50 microns).

Summary

Here we demonstrate growth of endothelial cell monolayers under gravity flow and an adhesion assay under flow with leukocytes and colon carcinoma cells using the Bioflux 200 system. We found that Jurkat cells adhered at higher numbers under flow when compared to HT29 cells, especially on activated monolayers. However, HT29 cells did undergo transient interactions with endothelial cells especially at breaks in the monolayer. All four conditions were assessed simultaneously on the same plate.