FLUXION

APPLICATION NOTE

Angiogenesis

Novel microfluidic assay for cancer cell invasion with real-time imaging

Introduction

Understanding the molecular and cellular mechanisms of cancer progression from primary malignancy to metastatic disease is critical to development of successful treatments. Cell invasion from the primary tumor and tumor-induced angiogenesis are just two of many phenomena contributing to the pathology of metastatic disease. Both biological processes involve migration and transmigration of cells in response to chemoattractants.

Conventional In vitro analysis of these phenomena typically involves deposition of a basement membrane derived matrix (i.e. Matrigel) in a static well plate, often times using a porous cell culture insert. These inserts can be difficult to process and often preclude capturing data in real-time. Here, we demonstrate a microfluidic method to follow angiogenesis and cell invasion in real time. The method enables acquisition of high content data by microscopy using microfluidic flow cells called BioFlux Plates. These devices are optimized for imaging and leverage the parallel flow capabilities of the BioFlux system to create a unique cell invasion environment (Figure 1).

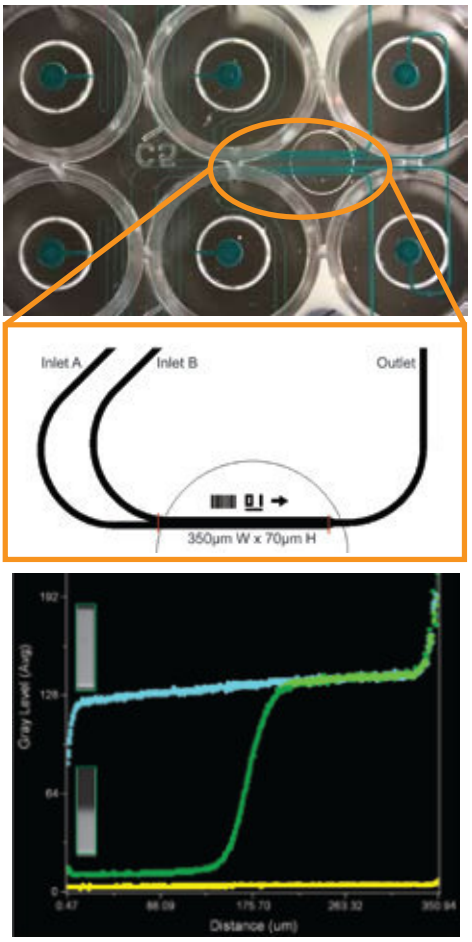


Figure 1: Creation of two microenvironments within a microfluidic channel is achieved using a flow channel with two parallel flow inputs.
A network of microfluidic channels are integrated into an SBS-standard well plate (top).
Each experimental zone has two fluidic inputs. When flowed under laminar fluid dynamic conditions, fluids originating from inlet A and B travel in non-mixing streams from beginning to the end of the channel (middle).
Laminar flow conditions were demonstrated by flowing fluorescent dye from one inlet and non-fluorescent buffer from the other while varying the pneumatic pressures on each well. Pressurizing the buffer well resulted in a channel with no fluorescence (yellow trace), while pressurizing the dye inlet resulted in a channel with all fluorescence (blue trace). Equal pressures applied to both inlets resulted in equal-sized zones of fluorescent dye and non-fluorescent buffer (green trace).

Application Overview

The microfluidic channels of the BioFlux 24-well plate were filled with matrix in one half of the channel while the other half was maintained in the fluid phase. Using this technique, we tested endothelial cell response to VEGF, bFGF and fumagilin. It was shown that VEGF and bFGF impregnated in the matrix promoted angiogenesis while fumagilin abrogated the effect. We also investigated invasion of serum starved HT1080 and MCF-7 cells into FBS containing matrix and found that, as predicted, only HT1080 cells successfully invaded the matrix. The method is amenable to screening multiple cell types, environmental conditions and compounds in parallel. High resolution microscopy data is produced which can be quantified to determine cell behavior and compound efficacy.

Materials and Methods

Please refer to the companion protocol for recommended experimental techniques.

A 24-well Bioflux device was chilled at -20°C for 10 minutes prior to preparation. Channels were primed with CO2-independent media (Invitrogen, Carlsbad,CA) supplemented with 1% FBS from the outlet well. After priming, the Bioflux device was placed on a flat wet ice pack. Undiluted Matrigel (BD Biosciences, Franklin Lakes, NJ) either alone or supplemented with VEGF(10ng/ml), bFGF (1ng/ml) or fumagilin(200 or 400 ng/ml) was added to the "B" inlet wells and media was added to the "A" inlet wells. Flow from both inlet wells was initiated with the device on the ice pack for a few seconds. Flow was continued with the device off the ice pack for 3 minutes; at the 3 minute mark, the device was placed on the glass-bottomed Bioflux heating plate preheated to 38°C. Flow was continued for 30 additional seconds. The perfusion was stopped with the plate on the heater and incubation at 38°C continued for 20 minutes. An additional incubation at room temperature was included to solidify the gel for 20 minutes (Figure 2).

Materials and Methods (cont.)

Following gel formation, the A-well was used to coat the remainder of the channel with 1/40 v/v diluted Matrigel in HBSS. The diluted Matrigel was perfused into the fluid space inside the channel. The device was incubated at 37°C for 30 minutes.

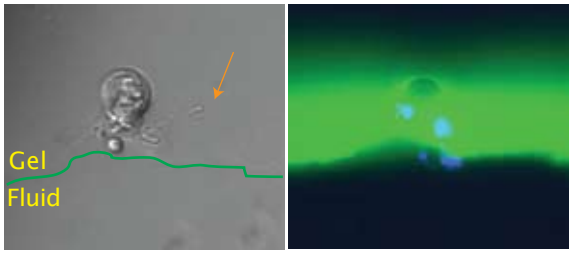
Cells were introduced into the plate from the A wells. Either P2 HUVEC, HT1080 colon carcinoma cells or MCF7 cells were added to the A wells at 1 x106 cells per ml. Cells were perfused into the main viewing window until an 30% density was reached. Flow was stopped. Cells were incubated for 1 hour without flow. After cells had attached, perfusion at 0.4 dyn/cm2 was commenced with 1% FCS supplemented CO2-independent media. For HT1080 and MCF7 cells, flow was continued for 15 hours under continuous timelapse. For endothelial cells, flow was continued for 36 hours.

Results

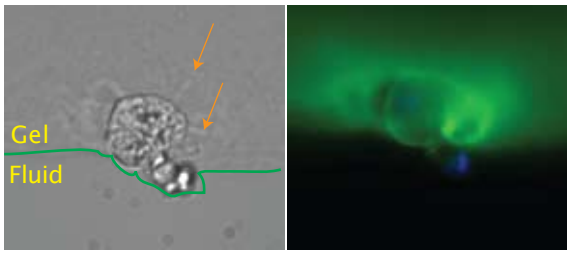
Endothelial cell matrix invasion is abrogated by fumagilin
HUVECs were grown under serum starvation conditions under 1 dyn/cm2 shear flow for 36 hours. Individual cells were observed invading the gel matrix under conditions of Matrigel alone, VEGF, bFGF. Sprouting and process formation, which is an early step of angiogenesis, was noted under these conditions as well. The addition of fumagilin, a angiogenesis inhibitor, abrogated the invasion and the cells multiplied outside of the gel (Figure 3).

Invasive carcinoma cells migrated into the matrix while non-invasive cells remained inactive.
Non-invasive cells (MCF7) and invasive cells (HT1080) were used to test cancer cell invasion into the Matrigel matrix. The cells were cultured overnight in the prepared BioFlux channels and imaged in time-lapse. The HT1080 cells began to migrate after 4 hours of perfusion into the matrix and continued invasion throughout the entire experiment. In contrast, the MCF7 cells were completely non-invasive, as expected (Figure 4).

Control Matrix



bFGF



Fumagilin (400ng/ml)

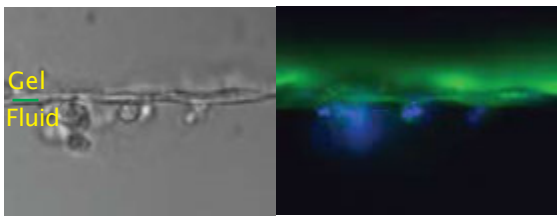


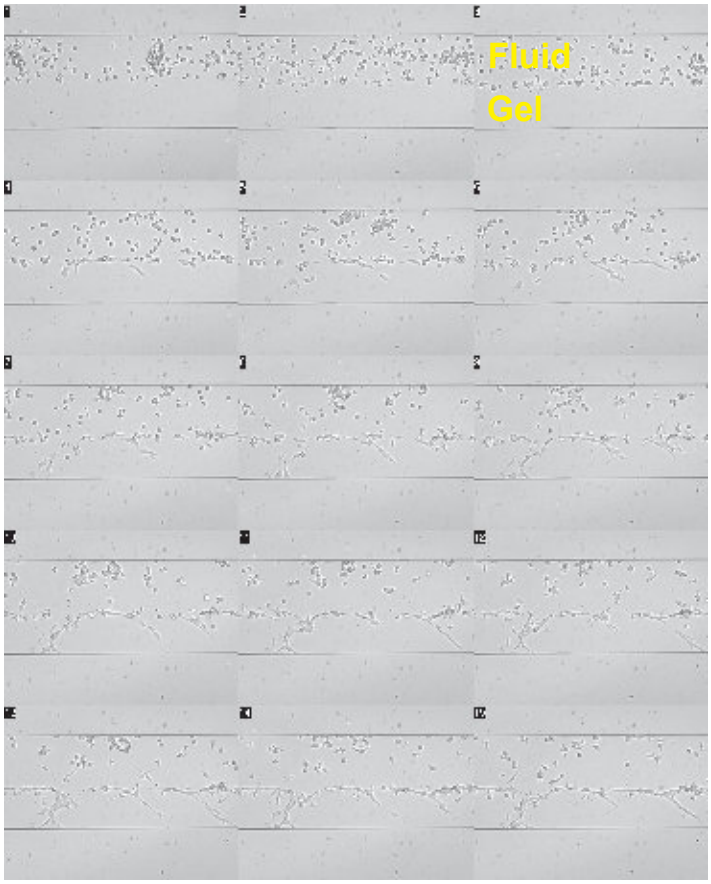
Figure 3. Invasion of endothelial cells into the Matrigel matrix. After 36 hours wheat germ agluttinen (green) was used to stain cells and gel for 15 min post-flow. Nuclei are stained with Hoescht 33342 (blue).

Summary

- Two inlet channels were used to create a side-by-side hydrogel (stationary) / fluid (under flow) environment.
- Under these conditions, cells were introduced and grown in the fluid side of the channel.
- Compounds introduced in the gel or the fluid created gradients in the opposite phase.
- Endothelial cells grown under flow responded by invading and beginning to sprout into the gel matrix. Fumagilin abrogated this effect.
- HT1080 cells were able to migrate and grow within the gel matrix while MCF-7 cells were not.

HT1080 Colon Carcinoma Cells

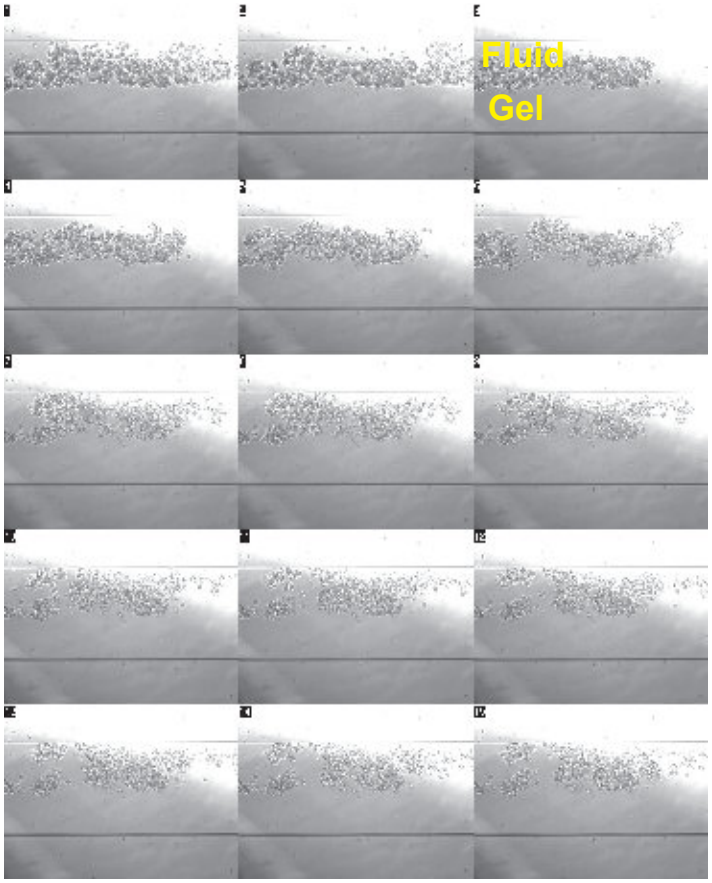
Time 0



Time 15h

MCF7 Breast Cancer Cells

Time 0



Time 15h

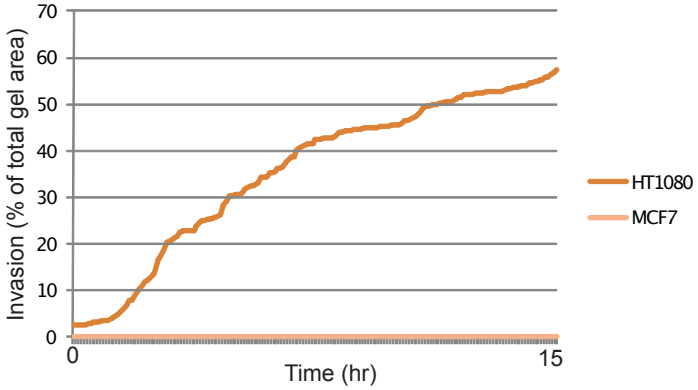


Figure 4. Cancer cell invasion into the Matrigel matrix. Timelapse over 15 hours of either invasive (HT1080) or non-invasive cancer cells (MCF7) into Matrigel (top). Cell invasiveness expressed as pixel intensity over total gelled area of the channel as a function of time (left).

FLUXION

(866) 266-8380 Toll Free
(650) 241-4777 Main
(650) 873-3665 Fax
www.fluxionbio.com