CELL Microsystems®

APPNOTE

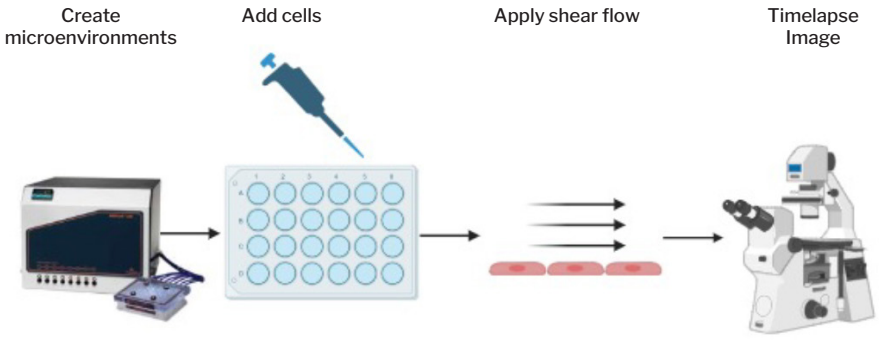
Cellular Migration and Invasion Assays

Real-time, dynamic measurements of cellular behavior with controlled microenvironments.

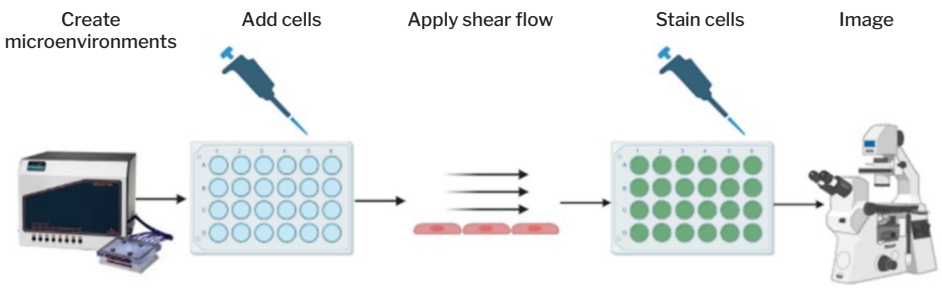
Introduction

Cellular migration is an important biological process in both health and disease. Cell migration along a chemical concentration gradient, termed chemotaxis, is vital for normal physiological processes, including wound healing, cell differentiation, immune responses, angiogenesis, and cancer metastasis. Chemical messengers, known as chemokines, induce numerous steps of cell communication and responses that direct cells to tissues. To examine detailed cellular migratory processes in a physiologically relevant manner over time, it is necessary to observe cells in situ. Unlike typical Boyden chamber or transwell migration assays, a BioFlux system allows for a broad analysis of the entire chemotactic process along with environmental control, while requiring considerably less hands-on time.

BioFlux Chemotaxis Workflow



BioFlux Angiogenesis & Invasion Workflow



Key Highlights:

1) Create dual microenvironments within a single microfluidic channel
2) Examine key aspects of the entire cellular movement process over time
3) High-throughput analysis of multiple compounds within the same experiment

Questions this App Note Answers

1. How can cellular movement assays, such as chemotaxis or invasion, be conducted using a BioFlux system?
2. What aspects of cellular movement can be observed and analyzed using a BioFlux system?
3. Can a membrane matrix, such as Matrigel, be used in a BioFlux plate?

Methods

Dual microenvironments within a channel

To demonstrate laminar flow conditions, two separate microenvironments were created within a single microfluidic channel. This was achieved using two inlet channels and parallel flow streams (Figure 1). First, a fluorescent dye was added to inlet A, followed by the addition of a non-fluorescent buffer to inlet B. Fluids were then flowed under laminar fluid dynamic conditions and the point where pneumatic pressure was applied was varied (Figure 2).

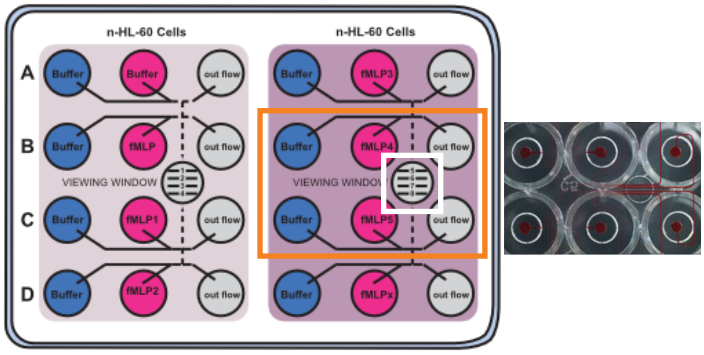


Figure 1. BioFlux plate channels viewed from beneath the well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

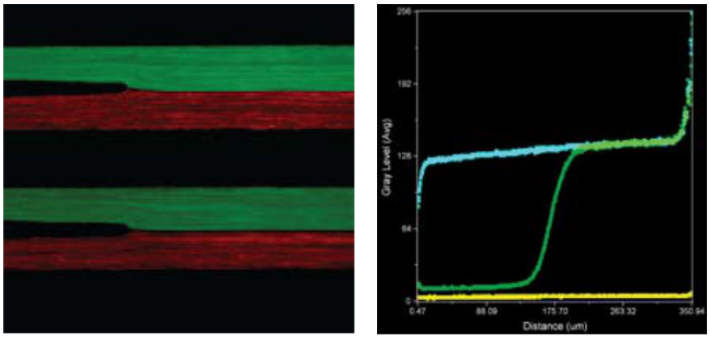


Figure 2. Microfluidic channels are shown (left) with different colored fluorescent beads flowing from separate inlets. Flow control provides the ability to fine-tune microcompartments (right). No pressure on the inlet with dye (yellow trace) or the inlet with buffer (blue trace) resulted in a channel with no fluorescence or all fluorescence. Equal pressures applied to both inlets (green trace) resulted in equal-sized zones of dye and buffer.

Table 1. Chemotaxis Plate Setup

Channel	Sample Type	Cell Type	Condition	
			Plate 1 fMLP [nM]	Plate 2 IL-8 [nM]
1	Control	n-HL-60	0	0
2	Exp	n-HL-60	2.5	0.0625
3	Exp	n-HL-60	25	0.312
4	Exp	n-HL-60	125	0.625
5	Exp	n-HL-60	250	1.25
6	Exp	n-HL-60	2500	12.5
7	Exp	n-HL-60	10000	6.25
8	Control	HL-60	250	1.25

Chemotaxis assay

n-HL-60 cells were differentiated into neutrophil-like cells using stimulation with growth factors prior to experimentation. HL-60 cells were left fully undifferentiated to serve as a control for chemotaxis. The experimental plate setup is shown in Table 1. Briefly, either N-Formylmethionyl-leucyl-phenylalanine (fMLP) or IL-8 was flowed from one inlet and buffer was flowed from the second inlet.

Cell chemotaxis was followed using timelapse microscopy. Image capture was performed using the BioFlux 1000Z imaging station. Data collection was 45 minutes, with an image captured every 15 seconds. Cells were counted using BioFlux Montage software at the beginning and the end of the experimental period. Cells were counted in the channel and in the regions of the channel where buffer and chemokine were present during the experiment.

Angiogenesis & Invasion Assay

The experimental layout of the angiogenesis and invasion assay is shown in Table 2. Leveraging the dual microenvironment, Matrigel, with or without treatments, was patterned into one side of the BioFlux channel and cells were added to the other. Treatments were either basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), or fumagillin (FUM).

Human umbilical vascular endothelial cells (HUVEC) p2, HT1080, or MCF7 cells were added. Buffer was flowed and after 36 hours, cells were then stained with wheat germ agglutinin (WGA) and Hoescht 33342 for 15 minutes. Timelapse images were captured for 15 hours. All samples were run in duplicate.

Data processing

Analyses were carried out using the Multidimensional Motion Analysis module in the BioFlux Montage software, using template matching. This module allowed for following many aspects of cellular movement over time, including distance, velocity, direction, angle, and many others.

Table 2. Angiogenesis and Invasion Plate Setup

Channel	Cell Type	Treatment	Channel	Cell	Treatment
Angiogenesis			Invasion		
1	HUVECp2	Matrigel (MG)	1	HT1080	MG
2	HUVECp2	MG+bFGF(1)	2	HT1080	MG+bFGF
3	HUVECp2	MG+bFGF(2)	3	HT1080	MG+VEGF
4	HUVECp2	MG+VEGF(1)	4	HT1080	MG+FUM
5	HUVECp2	MG+VEGF(2)	5	MCF7	MG
6	HUVECp2	MG+Fum(1)	6	MCF7	MG+bFGF
7	HUVECp2	MG+Fum(2)	7	MCF7	MG+VEGF
8	HUVECp2	MG	8	MCF7	MG+FUM

Table 2. Matrigel, with or without treatments added, was patterned into one side of the BioFlux channel; cells were added to the other. Timelapse data can be collected at any frequency for any duration desired.

Results

Chemotaxis

Chemotaxis results are presented as a percentage of the starting number of cells (Figure 3). Additionally, because shear flow removes some cells from the channel, results from the chemokine compartment are also presented as the percentage of total cells remaining at the endpoint (Endpoint %). Cells chemotaxed away from the buffer compartment into the chemokine compartment, indicated by a time-dependent increase in the percentage of cells in the regions where buffer and chemokine were present, expressed as a percentage of the starting number of cells (Chemokine compartment) and expressed as a percentage of total cells remaining at the endpoint (Endpoint %). The percentage of cells in the buffer compartment, expressed as a percentage of the starting number of cells (Buffer compartment), showed little change over time. Furthermore, chemotaxis toward fMLP was concentration-dependent, with a larger percentage of cells chemotaxing in response to higher concentrations of fMLP.

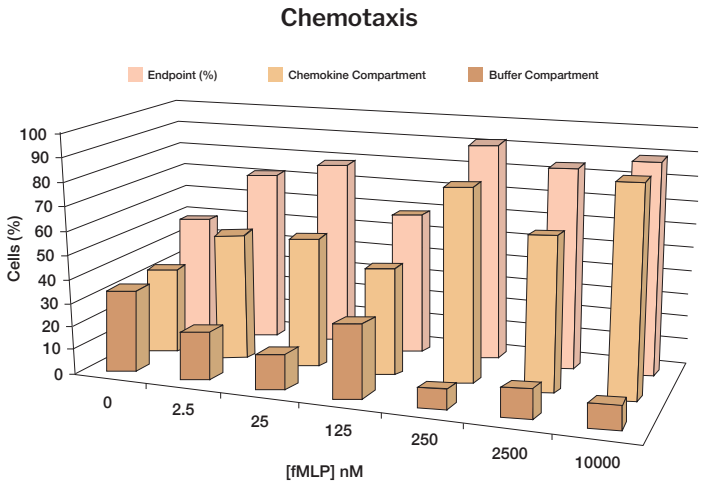


Figure 3. Chemotaxis results are presented as a percentage of the starting number of cells (Buffer + Chemokine compartments). In addition, because some cells are removed from the channel due to flow, results are presented as a percentage of total cells remaining at the endpoint (Endpoint %).

Angiogenesis & Invasion Assay

Individual cells were observed invading the gel matrix under conditions of Matrigel alone, VEGF, and bFGF (Figure 4). Additionally, sprouting and process formation, which are early steps of angiogenesis, were observed under these conditions. The addition of fumagillin abrogated invasion and cells multiplied outside of the gel.

HT1080 cells began to migrate into the matrix after 4 hours of perfusion. This invasion continued throughout the entire experiment. In contrast, MCF7 cells were completely non-invasive (data not shown).

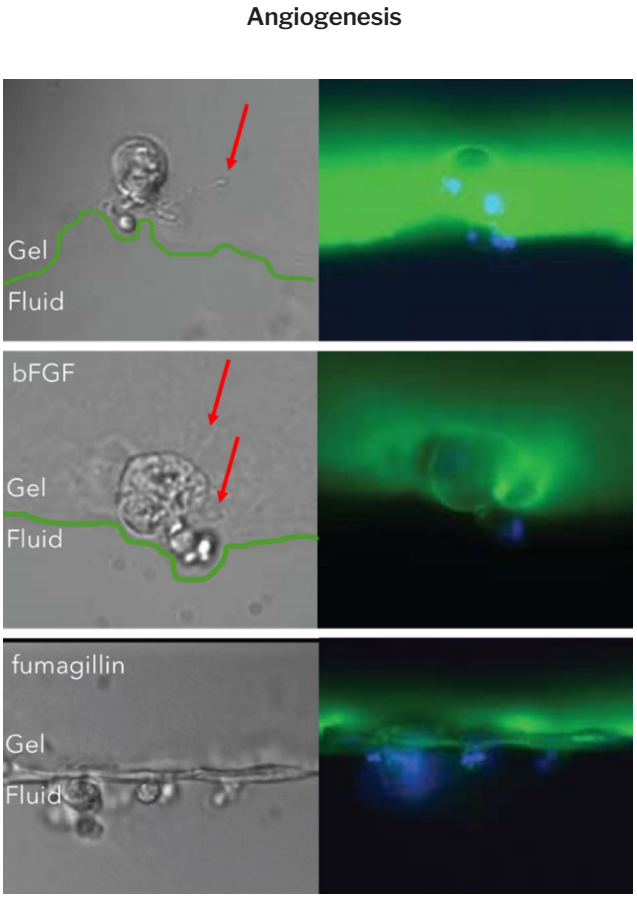


Figure 4. Individual cell invasion under conditions of Matrigel alone (top), VEGF (middle), and bFGF (bottom). Cells were stained with WGA (green) and nuclei were stained with Hoescht 33342 (blue). Sprouting and process formation (red arrows) were inhibited by fumagillin.

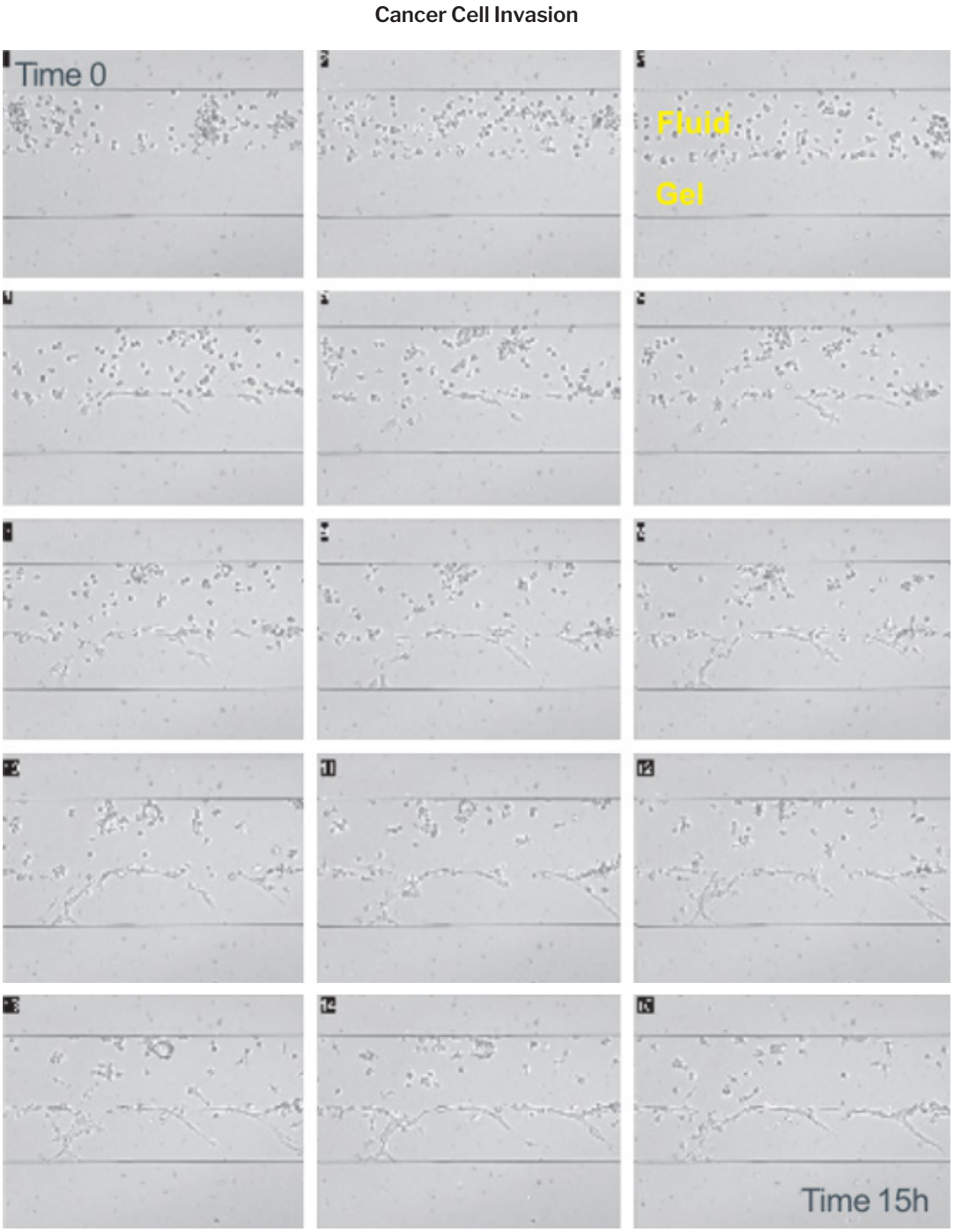


Figure 5. HT1080 cell invasion data presented as a timelapse over 15 hours.

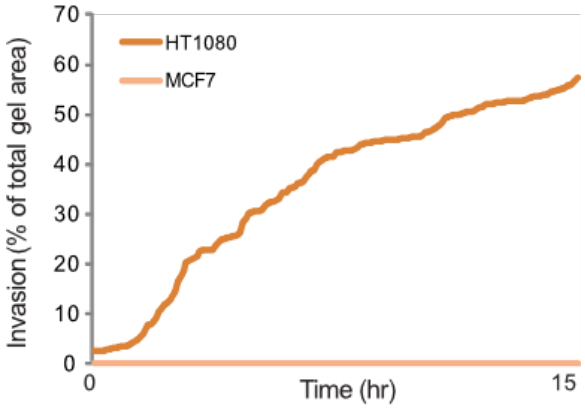


Figure 6. Cell invasiveness expressed as pixel intensity over total gelled area of the channel as a function of time.

Discussion

Using the BioFlux microfluidic system, we have demonstrated the creation of two independent microenvironments within a single microfluidic channel. This allowed for the in situ time-lapse assessment of chemotaxis, angiogenesis, and cellular invasion. Furthermore, when observing cells in the chemokine compartment, it is clear that some cells were lost during experimentation; however, the relatively small difference between chemotaxed cells expressed as a percentage of the starting number and expressed as a percentage of cells at the endpoint suggests that the number of cells lost was minimal. This highlights the usability of BioFlux plates for assaying precious cell types. The results of this investigation demonstrate the ease of conducting high-throughput chemotaxis assays, which can be leveraged for anti-inflammatory drug discovery applications and more.

Sprouting and process formation are key events in tissue invasion and survival processes of cancer cells during metastasis. These events were easily observable over an extended period. Combined with the high-throughput nature of a well plate, these abilities lend themselves well to the development of cancer treatments. Together with the BioFlux Montage software, the BioFlux system enables a wide range of conditions to be queried in parallel within a physiologically relevant environment.

For more information on the presented data or BioFlux Technology, visit cellmicrosystems.com

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