



LAB MODULE 2: Chemotaxis

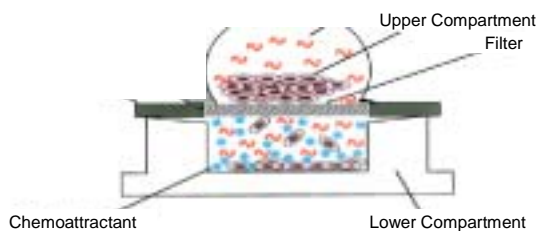
Purpose:

The purpose of this laboratory module is to provide an introduction and a hands-on demonstration of the use of microfluidic devices for generating chemical gradients at microscale and for cell motility studies. Microfluidic biochips with glass base and PDMS channels will be used to demonstrate neutrophil migration in the presence of a chemotactic gradient.

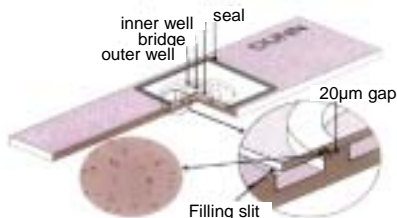
Overview of Chemotaxis:

The movement of cells is an essential function of critical importance throughout the lifetime of an individual, from embryogenesis and development to wound healing, inflammation or cancer. When cell movement occurs in response to chemical gradients of diverse substances, the process is called chemotaxis.

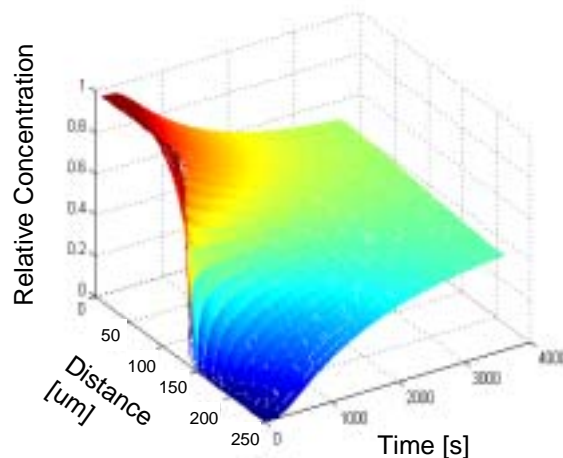
Current experimental techniques, while easy to use and thus widespread, have serious shortcomings in either the temporal or spatial control of the chemical gradient they can produce. Techniques relying on diffusion through filters, agarose, or collagen gels generate gradients that change in poorly defined ways in time and space (Fig.1) and, nonetheless, may be easily altered by the cells themselves.



Boyden Chamber



Dunn Chamber



Boyden chambers are the most frequently used devices for cell migration assays. However, they do not allow direct observation of cells, their output is semi-quantitative at best, and because of slow stabilization are impractical for studying transitory cellular responses. Shallow layer chambers, like the Dunn or Zigmond chambers, generate gradients that can stabilize in few

minutes, allow direct observation of cells, but, although changes of the chemotactic gradient are possible by replacing the solutions in the device, the convection currents formed while changing the chemicals can adversely affect the reproducibility of experimental conditions. Micropipettes can be positioned close to cells, turned on and off, or quickly displaced from one side of a cell to the other. However, the gradients produced are notoriously difficult to control and are easily perturbed by external factors. Moreover, the useful gradient generated around a micropipette is limited to a distance comparable to the cell size and repositioning of the micropipette is necessary in order to accommodate cell migration, further perturbing the gradients.

From an engineering perspective, microfluidics, working at the cell size scale, could provide new tools for controlled and reproducible chemical microenvironments to be established around cells. Recently, the spatial component of chemical gradients has been addressed by the development of microfluidic devices for generating stable gradients of active molecules in the cellular microenvironment.

Equipment, Materials, and Supplies:

- Microfluidic Biochips
- Syringe Pump
- Syringes, connectors, tubing (Tubing: TGY-010-C; needles NE-301-PL-C, Small Parts, FL – 1-800-423-9009 - www.smallparts.com)
- Inverted Optical Microscope with at-least a 40X objective (10X in eye pieces – total magnification 400X)
- Buffer
- Chemokine (fMLP – Sigma)

Module Outline and Workflow:

The two major objectives for this module are 1. to establish a chemical gradient at the microscale using the microfluidic device 2. to observe cell migration in the presence of a chemotactic gradient.

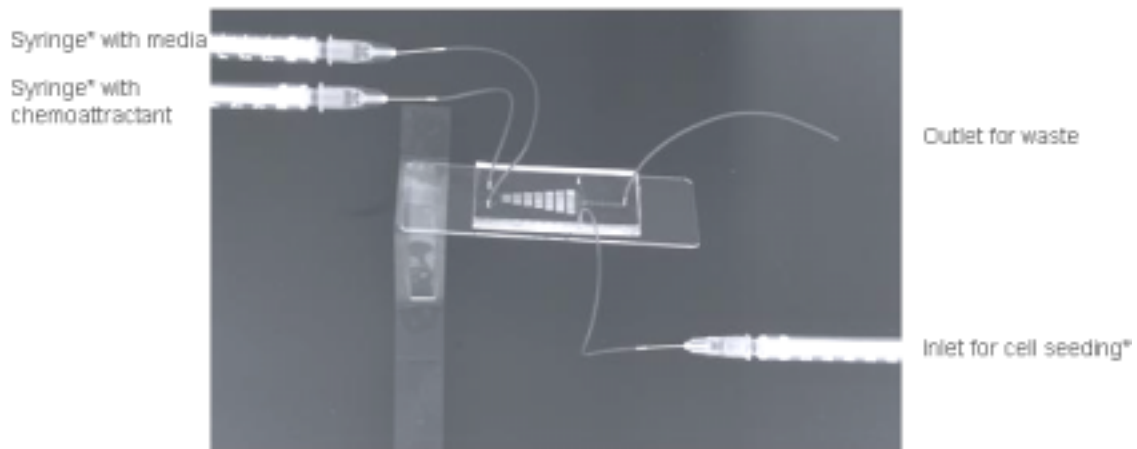
1. Microfluidic setup:

The overall microfluidic setup is shown in Figure 2. The most critical thing in setting up the device is to avoid the trapping of air bubbles in the microchannels. Because of capillary effects, any air bubble in the small channels will perturb the flow and thus the gradient. For this we will first fill the whole device with buffer (e.g. PBS) by disconnecting all syringes and connecting a 5mL syringe with buffer at the outlet. We will push the liquid slowly until the syringe needle at the cell inlet is completely filled up with liquid. Then, we will use a pair of forceps to clamp the tubing between the device and the cell inlet. We will keep pushing buffer in until the two other syringe tips at the two inlets (for media and chemoattractant) are completely filled up. After clamping the two tubing using two forceps, we will keep applying moderate pressure on the buffer syringe under the microscope until all the bubbles in the device shrink and disappear. In the end, we will scan the whole device under the microscope looking for bubbles and also check at the inlets, where the tubing is connected to the device.

To create the gradient, we will fill one syringe with media, and one with the maximum concentration of chemokine you need for the experiment. We will mount the two syringes in a

syringe pump and set the flow rate to 1 μ L/min. We will manually push the pump until there is no air in the syringes, only liquid and connect the syringes with the syringe needles, taking care not to trap any air bubble. After detaching the forceps and starting the pump we will wait for several minutes until all the buffer is replaced by media and chemokine.

Overall view of the assembled chemotaxis device



*These two syringes should be mounted in a syringe pump set to 0.1-1 μ L/min

*This syringe is only used for introducing the cells into the main channel after which is locked.

2. Cell migration

We will check the formation of a stable gradient by using a fluorescent dye introduced with the chemokine. We will then introduce the cells. For this, we will clamp the inlets again, stop the pump, and release the clamp on the cell inlet tubing and carefully push the cells in, either directly from a syringe, or after loading them in the syringe needle using a micropipette. After cells are attached to the glass in the main channel, we will release the clamps on the inlet tubing and start the pump again to reestablish the gradient. A linear, stable gradient forms in 20-40 seconds, depending on the flow rate.

Related References:

1. N. L. Jeon, H. Baskaran, S. K. W. Dertinger, G. M. Whitesides, L. Van De Water and M. Toner. Neutrophil chemotaxis in linear and complex gradients of Interleukin-8 formed in a microfabricated device. *Nature Biotechnology* 20, 826 - 830 (2002)
2. D. Irimia, S-Y Liu, W. G. Tharp, A. Samadani, M. Toner, M. C. Poznansky, Microfluidic systems for measuring neutrophil migratory responses to fast switches of chemical gradients. *Lab-on-a-chip* 2006;6:191-198.
3. W. G. Tharp, A. Upadhyaya, P. Yadav, D. Irimia, A. Samadani, O. Hurtado, S-Y Liu, S. Munisamy, D. M. Brainard, M. J. Mahon, O. Nourshargh, M. Toner M, M. C. Poznansky, Neutrophil chemorepulsion in defined interleukin-8 gradients in vitro and in vivo. *J Leukocyte Biology* 2006;79:1-15.
4. D. Irimia, D. Geba, M. Toner, Universal microfluidic gradient generator. *Analytical Chemistry* 2006; 78:3472-3477.