



LAB MODULE 3: Cell Capture in Biochips

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Purpose and Expected Outcome:

The purpose of this laboratory module is to provide an introduction and a hands-on demonstration of cell capture in a microfluidic chip. Antibody-functionalized biochips comprising glass bases and PDMS channels will be used to demonstrate trapping of a specific type of cells from whole blood under a controlled flow condition. The students will be able to examine the interactions between blood cells and surface immobilized antibodies. The expected outcome is for the students to gain a basic understanding of cell affinity chromatography in a microfluidic format and its potential applications for biology and medicine.

Overview of cell capture in a microfluidic channel:

The isolation of phenotypically pure subpopulations from a cell mixture, such as whole blood, is important in both clinical diagnostics and basic research. The use of antigen tags on cells is a common way to separate cell subpopulations from a cell mixture. By immobilizing antibodies on a substrate, cells expressing the corresponding antigens can be isolated on the surface without the requirement of pre-labeling. This is the principle behind cell affinity chromatography. Compared to conventional packed bed design, an antibody-functionalized microfluidic channel offers the advantage of high surface area to volume ratios with short residence times for cell isolation.

Effective cell capture in a microfluidic channel requires specific surface chemistry and controlled flow condition^{1,2}. To minimize non-specific cell binding from whole blood, antibody immobilized surfaces are usually blocked with PEG or albumin. Fluid is delivered into microfluidic devices at desired shear conditions using pumps and/or pressure regulators. The effect of shear stress on captured CD4+ T cell density is shown in Figure 1.

Equipment, Materials, and Supplies:

- Microfluidic biochips
- Syringe pump
- Syringes, connectors, tubing
- Fluorescent antibodies for cell identification
- Inverted fluorescence microscope
- Buffy coat (or whole blood)

Module Outline and Workflow:

Microfluidic devices functionalized with anti-CD4 antibody will be prepared beforehand (Figure 2). Ten microliter of buffy coat (or whole blood) from healthy donors will be flowed into the biochip at 5 $\mu\text{l}/\text{min}$. After rinsing at a flow rate of 20 $\mu\text{l} / \text{min}$, cells adhered on the surface will be stained with an antibody mixture containing AF488-anti-CD4 and PE-anti-CD14 (a monocyte marker for check non-specific binding in the channel).

The students will help prepare the tubing and operate the syringe pump. After staining, students will observe captured cells under fluorescent microscope.

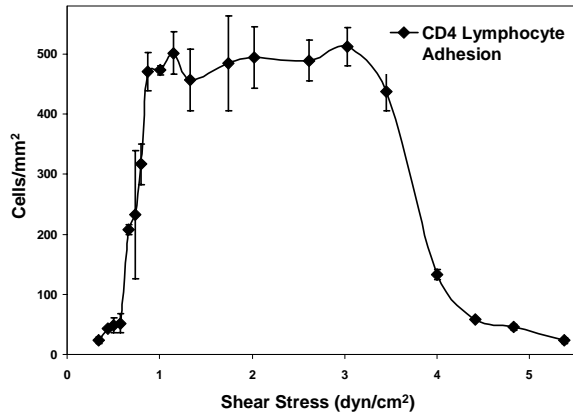


Figure 1. Effect of shear stress on cell using whole blood from healthy subjects. A shear stress window between 1 and 3 dyn/cm² was optimal for CD4+ T cell adhesion.

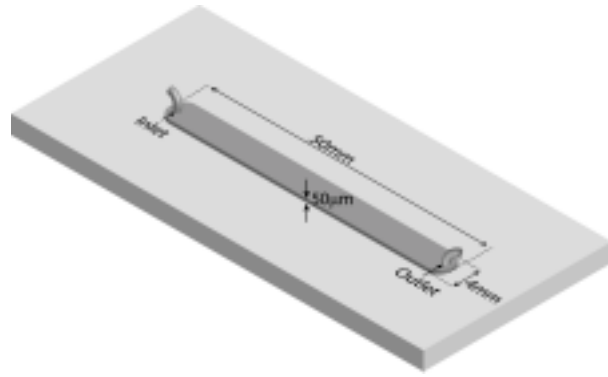


Figure 2. Geometry of a cell capture device. Microfabricated PDMS devices with one inlet and one outlet were bound to glass slides and functionalized with specific antibody to capture target cells from whole blood.

Related References:

1. Murthy, S. K., Sin, A., Tompkins, R. G. & Toner, M. Effect of flow and surface conditions on human lymphocyte isolation using microfluidic chambers. *Langmuir* 20, 11649-11655 (2004).
2. Sin, A., Murthy, S. K., Revzin, A., Tompkins, R. G. & Toner, M. Enrichment using antibody-coated microfluidic chambers in shear flow: Model mixtures of human lymphocytes. *Biotechnology and Bioengineering* 91, 816-826 (2005).