MICROFLUIDIC ANTIBODY MICROARRAY WITH AN ELECTRONIC READOUT FOR COMBINATORIAL IMMUNOPHENOTYPING OF CELL POPULATIONS

Ruxiu Liu, Chia-Heng Chu, Ningquan Wang, A. Fatih Sarioglu
Georgia Institute of Technology, USA

ABSTRACT

Immunophenotyping of heterogenous cell populations has important research and clinical applications. In this paper, we introduce an antibody microarray with an electrical readout for label-free combinatorial immunophenotyping of cell populations. Our device consists of microfluidic cell capture chambers pre-functionalized with antibodies against target antigens and code-multiplexed Coulter counters placed at strategic nodes across the device to quantify the fraction of cell population captured in functionalized chambers. Through combinatorial arrangement of antibody sequences along microfluidic paths, our device achieves all-electronic combinatorial immunotyping of cell populations against multiple antigen targets. Our device was tested with human blood, and estimated prevalence of different leukocyte subpopulations were shown to agree with results from a commercial hemocytometer.

KEYWORDS: Immunophenotyping, Lab on a chip, Microfluidic CODES, Surface modification, Cell capture

INTRODUCTION

Immunophenotyping (i.e., identifying the membrane protein expression, usually CD antigens, of cells) is widely used to characterize cells in lineages of differentiation from hematopoietic stem cell, and also to diagnose and classify diseases derived from those cells. This process typically requires flow cytometers or fluorescence microscopy, which are typically housed in centralized laboratories. While microfluidic devices have been used as immunophenotyping assays through immunoaffinity-based capture of the target cells, these devices either required external tools for readout [1] or were limited to a single antigen type [2]. Here, we present a method for combinatorial immunophenotyping of cells against multiple antibodies in a microfluidic system, that readily provides quantitative results as an electrical signal. Our approach is based on the integration of multiple cell capture chambers, each selectively functionalized with a specific antibody, with Microfluidic CODES [3], a multiplexed electrical sensor technology to quantify the results of the assay.

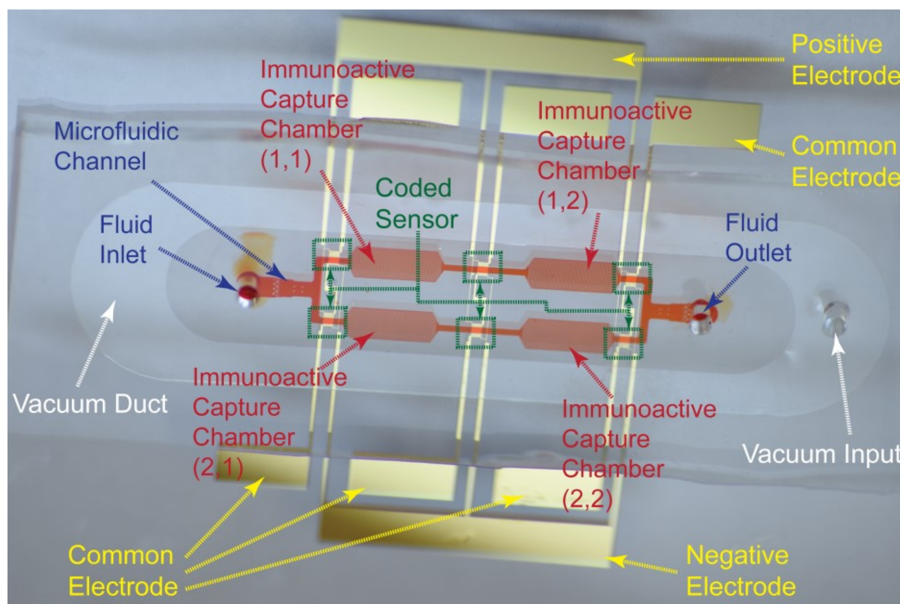

Figure 1. A photo of the whole microfluidic device for electronic combinatorial immunophenotyping of cells. Four microfluidic chambers consisting of micropillars are functionalized with different antibodies. For each chamber, differential capture rate is monitored using code-multiplexed sensors placed at strategic locations.

THEORY

We designed and fabricated a proof-of-concept device with a 2×2 capture chamber array, each functionalized by a specific antibody (Figure 1). The microfluidic chambers were arranged as two parallel tracks, with each track consisting of two serially connected chambers. In each chamber, micropillars were used to increase the cell capture efficiency. Code-multiplexed electrical sensors encoded with distinct digital codes were placed at strategic locations across the device to quantify the capture rate in each chamber through differential counts. All of the sensor data is collected from a single waveform, which was computationally analyzed to obtain immunophenotyping results. Our device is fully integrated yet frugal, consisting of a polydimethylsiloxane (PDMS) microfluidic channel layer fabricated using a soft lithography process, and an electrode-patterned glass substrate fabricated with a lift-off process.

EXPERIMENTAL

To functionalize our device, we developed a new technique to selectively modify individual capture chambers (Figure 2). Briefly, prior to bonding, the PDMS layer was first treated with (3-Mercaptopropyl)-trimethoxysilane solution droplets for 1 hour, followed by incubation with N-y-maleimidobutyryloxy-succinimide-ester (GMBS) for 30 minutes and NeutrAvidin solution for 1 hour. After washing and gentle drying, the PDMS layer was aligned and reversibly attached to an auxiliary PDMS layer consisting of microscale features that isolate individual chambers by sealing the fluidic channels in between. The auxiliary component also had pre-punched holes above the chambers and allowed different biotinylated antibody solutions to be introduced and selectively incubated in different chambers for 1 hour. Finally, the PDMS layer was sealed with the glass substrate using vacuum [4], and primed with bovine serum albumin to block non-specific cell adhesion.

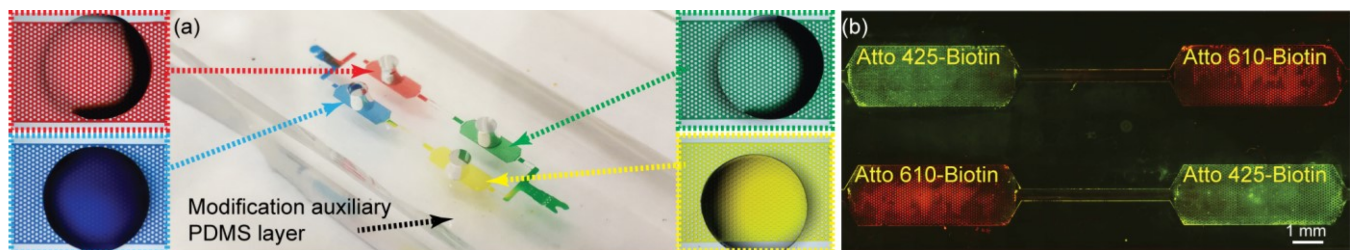

Figure 2. Functionalization of the microfluidic capture chambers. (a) A photo shows the microfluidic chambers selectively filled with different-colored dyes with the aid of an auxiliary PDMS layer. (b) A fluorescent image of a device with its chambers independently labeled with biotinylated Atto 425 and Atto 610 with a 2 by 2 checkerboard pattern. Results clearly demonstrate minimal cross-contamination between chambers.

We tested the device with human blood sample following the lysis of red blood cells. The chambers of the device were pre-treated with four different antibodies (anti-CD66b, anti-CD15, anti-CD38, and anti-CD45 antibodies) (Figure 3a), and the sample was driven through the device using a syringe pump. A 500 kHz sine wave was applied to drive the sensor network, and signals from the sensors were measured using a lock-in amplifier.

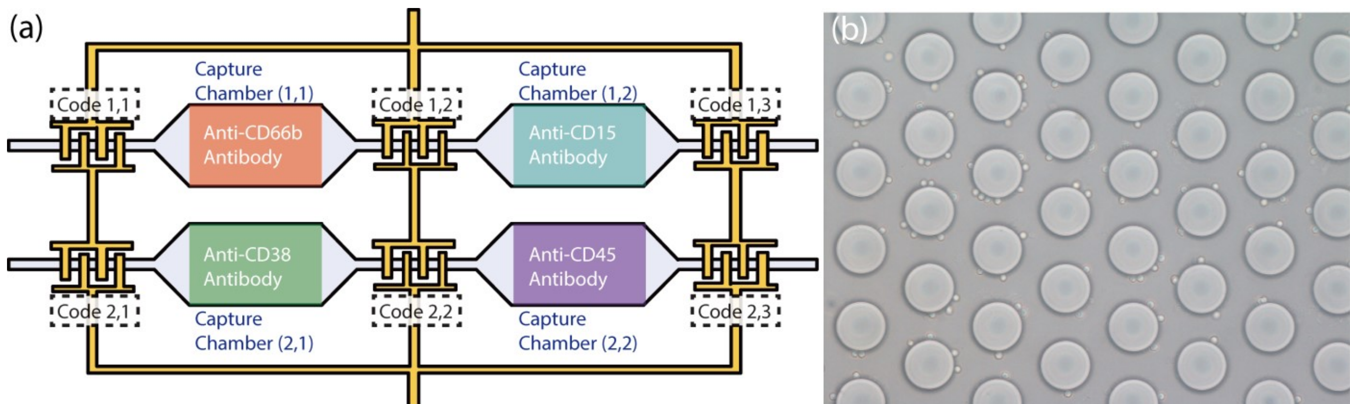

Figure 3. (a) Schematic showing the arrangement of antibodies anti-CD66b, anti-CD15, anti-CD38, and anti-CD45 in the microfluidic device. (b) Cell capture in functionalized microfluidic channels were validated using microscopy.

RESULTS AND DISCUSSION

Cell capture in different microfluidic chambers was independently validated with microscopy (Figure 3b). Subpopulation fractions estimated from the sensor data (Tables 1&2), mostly agree with the complete blood cell analysis of the sample using a commercial hemocytometer (Abbott, CELL-DYN Ruby).

Table 1. Counts from each coded electrical sensor

Coded sensor number	Particle count
Code 1,1	2541
Code 1,2	1015
Code 1,3	574
Code 2,1	576
Code 2,2	356
Code 2,3	305

Table 2. Percentage of leukocyte subpopulations obtained using our device

Chamber number	Immunophenotype	Percentage	Cell type
1,1	$CD66b^{+}$	60.0%	Granulocytes
1,2	$CD66b^{-}$ $CD15^{+}$	17.4%	Monocytes
2,1	$CD38^{+}$	38.2%	Lymphocytes
2,2	$CD38^{-}$ $CD45^{+}$	8.85%	Other leukocytes

CONCLUSION

We introduced a microfluidic antibody microarray with a direct electrical readout, based on the Microfluidic CODES sensor technology. Our technology provides an integrated platform for label-free combinatorial immunophenotyping of cell populations. Experiments with blood samples demonstrated the feasibility of using the reported device for immunophenotyping of leukocyte subpopulations.

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CONTACT

* A. F. Sarioglu; phone: +1-404-894-5032; sarioglu@gatech.edu