Development of Microfluidic Devices for Use in Immunophenotyping

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Abstract:

Immunophenotyping presents significant promise in the diagnosis and prognosis of immune system disorders. While a means of detection of diseases including HIV/ AIDS, tuberculosis and sepsis, current methods (flow cytometry and enzyme-linked immunosorbent assay or ELISA), lack specificity, as well as time and sample-volume efficiency. Microfluidic devices may present the solution to such limitations. Through the integration of a high-porosity, polydimethylsiloxane (PDMS) microfiltration membrane (PMM), with a microfluidic microfiltration platform, microbead filtration may be used to achieve antigen-specific cellular isolation. The PMM was fabricated via semiconductor microfabrication techniques including traditional lithography and reactive ion etching (RIE). The silicon molds for components of the microfiltration platform were fabricated using photolithographic techniques, followed by deep reactive ion etching (DRIE). The PDMS components were then fabricated via soft lithography and layered to construct a complete device for microfiltration and chemiluminescence assay application for highly sensitive subpopulation characterization. This microfluidic device may drastically reduce time, costs and inaccuracies of immunophenotyping, yielding more efficient detection, evaluation, and treatment of disorders.

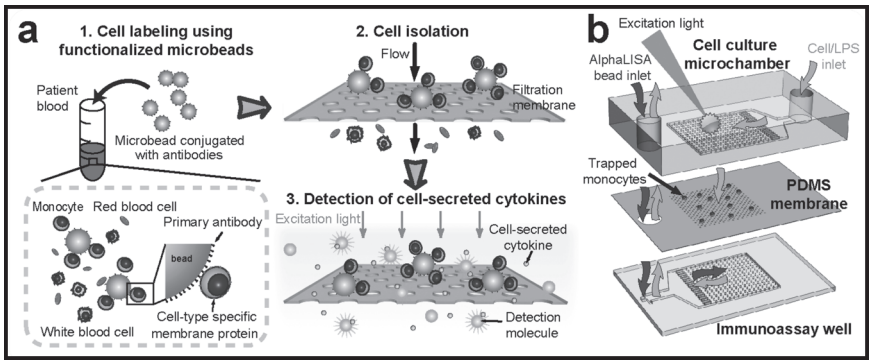

Figure 1: The proposed PMM-integrated device.

Introduction:

The immune system has various leukocyte constituents within the blood, maintaining particular functions in disease combating. Infections and other obstructions cause abnormalities amongst constituents, characterized in the numbers, proportions or functional responses of leukocyte subpopulations. For example, human immunodeficiency virus (HIV) causes $CD4^+$ T cell depletion. Thus, the identification of disproportionately-low $CD4^+$ T cells and overall T-cell proportions provide an indication of acquired immunodeficiency syndrome (AIDS) [1]. Immunophenotyping technology is used to identify qualitative abnormalities in immune cell subpopulations for use in diagnosis and prognosis. The current challenge in the design of immunophenotyping devices is overcoming the time, cost, and sample-size inefficiencies, while increasing characterization specificity. A microfluidic device is proposed with the integration of a high-porosity PMM within a microfluidic chamber, consisting of cell culture and immunoassay components (see Figure 1). The PMM is designed for efficient bioparticle separation. Microbeads are coated with cell-specific antibodies, resulting in the selective capture of the cell subpopulations via bonding of cell surface proteins to beads. Pore diameters are designed to trap only bead-subset groups above the PMM. The PMM was fabricated with traditional lithography and RIE [2], then layered between microchamber components [1]. This device will isolate cell subsets and characterize populations using a no-wash chemifluorescence assay (AlphaLISA) [3].

Experimental Methods:

PMM Micromachining. PMM fabrication was completed using a surface micromachining technique for soft materials [2]. A silicon wafer was O_2-plasma activated and silanized with (tridecafluoro-1,1,2,2,-tetrahydrooctyl)-1-trichlorosilane vapor for later PMM release. The PDMS prepolymer was prepared with a 1:10, curing agent: base monomer ratio, spun onto silanized wafers at 7,000 rpm for 30 s, then cured at 60°C overnight. The PDMS surface was plasma activated for 5 min, with coating of AZ 9260 photoresist immediately following. A soft-bake process at 90°C for 10 min and traditional exposure

and development followed. The wafers were processed with RIE using SF_6 and O_2 mixtures to anisotropically pattern PDMS layers.

Device Construction. The device consisted of cell culture and immunoassay layers fabricated from silicon molds made via photolithography and deep reactive ion etching (DRIE) [1]. The cured PDMS structures sandwiched the PMM atop a glass slide via a O_2 plasma-assisted bonding process.

Device Testing / Functional Immunophenotyping. Device testing included capture efficiency of the PMM, quantified with ImageJ. The cultured cells were labeled and captured according to previous protocol [1]. Microbeads and cells were quantified via hemocytometer, then imaged via fluorescence microscopy (see Figure 4). After microfiltration of cells, LPS addition induced cytokine secretion. AlphaLISA signal detection was facilitated by AlphaLISA bead interaction with cytokines [1, 3].

Results and Discussion:

The PMM was successfully fabricated and integrated into the device (see Figures 2 and 3). Some membranes were corrupted during the micromachining process due to separation between photoresist and PDMS layers following exposure. Moderate success rates during PMM fabrication suggest a need for improvement in the micromachining process, including an adjustment in soft-baking or plasma-activation steps to ensure adequate photoresist-PDMS annealing. High capture purities (~95%, whole and lysed) confirm that the PMM successfully filters cells. Percentage of monocytes were at 4% and 6% for whole and lysed blood, respectively, within the range of $CD14^+$ monocyte content for a healthy individual (see Figure 4), confirming that the correct subpopulation was isolated. The multi-component device facilitates multi-step processes with high specificity and reduced time (10-fold reduction), sample volume (5 μL / assay) and effort of processes, confirming it as a superior immunophenotyping device.

Conclusions:

We successfully fabricated a PDMS-based, bioparticle separation membrane that is easily integrated into a microfluidic, immunophenotyping device. Immune cells were isolated and characterized for use in functional immunophenotyping. The testing of microfiltration capabilities revealed that the membrane had a high capture yield and capture purity. The same device yielded successful characterization of immune cells via AlphaLISA biosensing with a vast reduction in process time. With the shortened assay time, heightened sample efficiency, and the ability to determine the functional status of subpopulations of immune cells, the PMM-integrated device is a novel approach to diagnosis and prognosis of immune diseases and disorders.

Acknowledgements:

Research was supported by the National Science Foundation (NSF) and the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program. Further thanks are offered to the Integrated Biosystems and Biomechanics laboratory at the University of Michigan, Ann Arbor, and the Lurie Nanofabrication Institute (LNF).

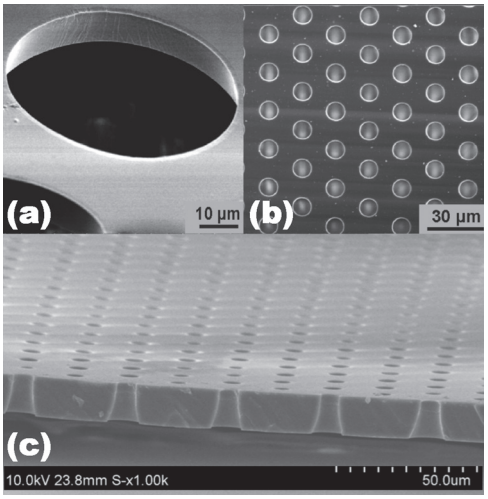

Figure 2: SEM views of the PMM, including above-membrane (a) and (b), and cross-sectional (c).

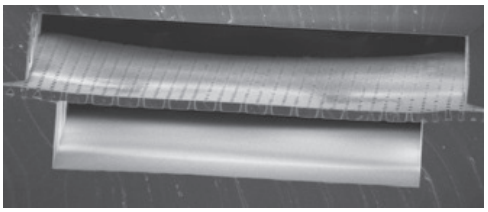

Figure 3: The complete device, with PMM between chamber components.

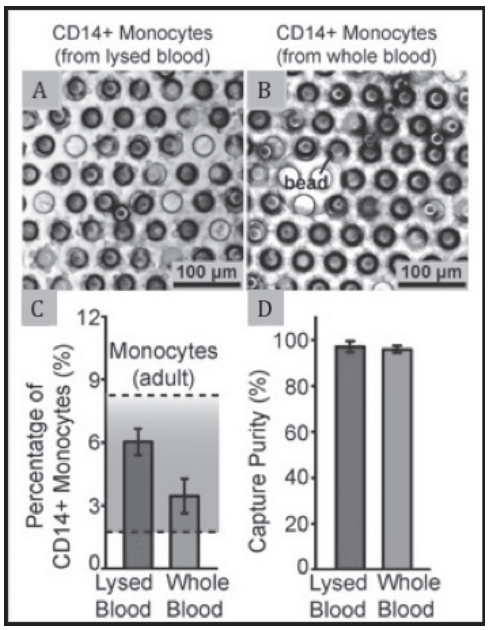

Figure 4: Images of monocyte capture from lysed and whole blood (A and B), monocyte percentages and capture purity for samples (C and D).

References:

[1] Chen, W., et al., Material Views, 1-11, 2013.
[2] Chen, W., et al., Lab Chip, 12, 391-395, 2012.
[3] Bielefeld-Sevigny, M., Assay Drug. Dev. Technol., 7, 90, 2009.