Nanoplasmonic Biosensing Microfluidics for Immune Status Monitoring of Critically-Ill Children

Tayler Pauls

Biomedical Engineering and Physics, University of Arkansas

NNIN REU Site: Lurie Nanofabrication Facility, University of Michigan, Ann Arbor, MI

NNIN REU Principal Investigator: Dr. Katsuo Kurabayashi, Mechanical Engineering, EE and CS, University of Michigan

NNIN REU Mentor: Dr. Robert Nidetz, Mechanical Engineering, University of Michigan

Contact: tpauls@uark.edu, katsuo@umich.edu, nidetz@umich.edu

Introduction:

The availability of a real time immune system monitoring system can be extremely useful in the laboratory and has potential clinical uses. The current standard for monitoring is an enzyme-linked immunosorbent assay (ELISA), which takes 1-2 days and requires large amounts of sample in comparison to a microfluidic system. Using a microfluidic system that can detect and monitor the activity of immune system in real time has many promising applications. Monitoring specific cytokines, which are proteins expressed from white blood cells that are triggered, and trigger the immune system, provides information as to the current state of the immune system. Information gathered from the microfluidic assay based on the cytokines can aid physicians to determine the best possible treatment and can have near immediate updates of the patient's state. This can prove extremely useful in immune-status updates pre-and post-operation, as well as a system of monitoring during an infection or disease. Single cell capture enables studying the most basic unit of our bodies and can potentially transform treatment and understanding of diseases.

Recently, the Fu group incorporated micropipette aspiration as a mechanism to study the mechanical differences of cancerous versus non-cancerous cells and the consequences for future cancer treatments [1].

The device proposed here aims to capture single cells to monitor their isolated behavior for relevant time periods for critical immune status updates. The microfluidic device consists of distinct chambers for single cell capture (see Figure 1). A cross-linked, biocompatible, synthetic polymer, polyethylene glycol (PEG-gel), will be patterned on top of the chambers to enable cell capture. In conjunction with the PEG-gel pattern, the previously developed technology of localized surface plasmonic resonance (LSPR) of gold nanorods can be implemented for biosensing secreted proteins [2].

Experimental Procedures:

CAD and Device Fabrication. K-layout was used for the design of the microfluidic device. Two designs were ultimately used in our process. The first design consisted of an inlet, outlet, and three arrays of cell capture chambers throughout the channel, varying with different sizes of the chambers per device, see Figure 1. A silicon wafer was fabricated following the device design (Figure 2) using standard DRIE procedures. The second design consisted of holes displaced relative to the center of the cell capture chambers in the first design, which was used to pattern PEG-gel.

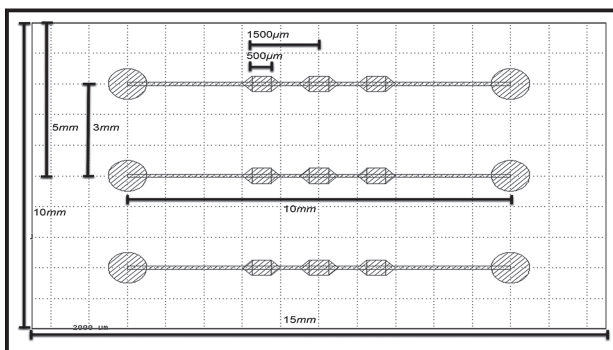

Figure 1: A CAD drawing of the device design that is used for PDMS molding. Dimensions not listed are; channel width of 100 mm, and channel depth of 50 mm. Each channel has three cell capturing wells.

Figure 2: Images of the fabricated silicon wafer. (a) Entire channel. (b) Cell capture chamber. (c) 3-D image of (b) to show topography.

Soft Lithography. PDMS and glass substrates were activated via oxygen plasma treatments prior to silanization. Polyethylene glycol diacrylate, MW 575 g/mol, was spun on the samples and exposed over the mask via UV-light for 2-3 seconds. After development by deionized water, the samples were dried by nitrogen gun.

Cell Capture. Human T-Helper cells (THP-1) were used in conjugate with CD14 primary antibody. First, the antibody was deposited and dried onto the features. After a sufficient drying time, at least 30 minutes, a PDMS microchannel was placed over the features and THP-1 cells concentrated to 10^5 cells/mL are flown through the channel. Approximately every ten minutes, for 30 minutes, 10 ml of cells were manually pumped via micropipetting, see Figure 3.

Data Acquisition. All cell capture experiments were performed with a Nikon Eclipse Ti-S microscope under brightfield view. Fabrication images were taken by an Olympus BX-51 microscope under bright view.

Results and Conclusions:

Soft lithography and cell capture proved successful on both PDMS and glass samples, but poor surface adhesion of PEG-gel onto PDMS substrates limited PDMS patterned PEG-gel experiments. Varying the exposure time, oxygen plasma treatments, silanization baths, and development times with PDMS patterning did not substantially improve the lithography. Swelling of the PDMS during the silanization step proved to be quite difficult to overcome for consistent or accurate patterning. Therefore, most cell capture experiments were carried out with PEG-gel features on glass slides.

However, cell capture was successful on a large area of PEG-gel on a PDMS sample. After many trial and error experiments, favorable antibody dry time coupled with sufficient cell flow exposure to the features was established. Single cell capture has not yet proved successful on the tested 50 mm diameter PEG-gel features, but double cell capture has (see Figure 4). Lithography of the 25 mm PEG-gel features have not yet been detected on the glass slide substrate.

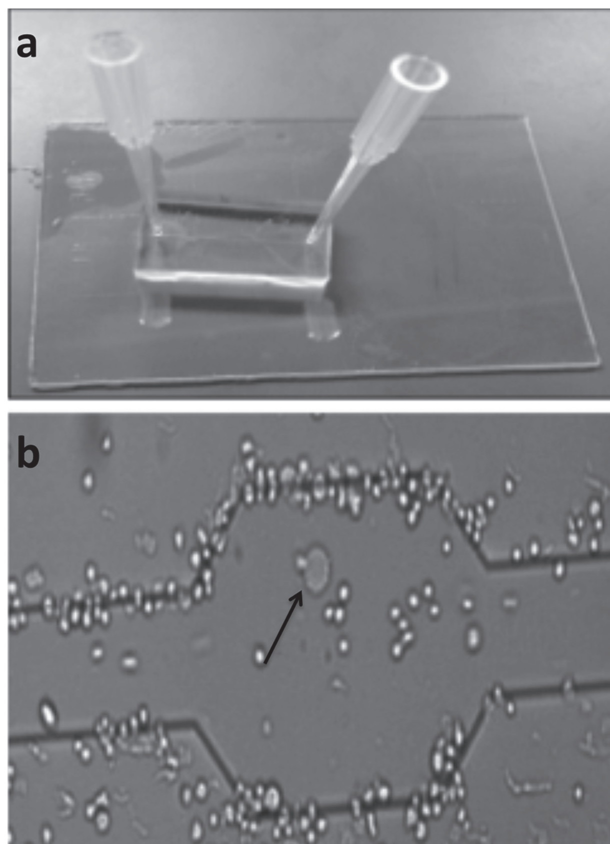

Figure 3: (a) Cell capture experimental set up. (b) Cell capture experiment, arrow pointing at PEG-feature patterned on glass.

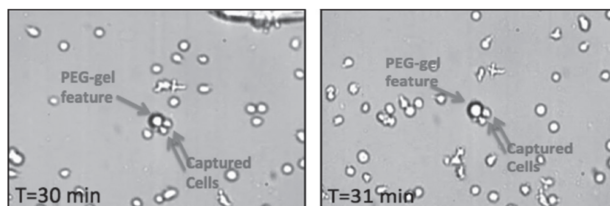

Figure 4: Time lapse demonstrates two cells captured on the PEG-gel feature after 30 and 31 minutes.

Future Work:

With double cell capture being possible onto the PEG features, more efforts will be employed for successful lithography onto PDMS including the use of a dessicator chamber for vapor silanization to avoid the swelling due to the solvent used. An addition, altering the cell capture design may be a possibility to improve the efficiency of cell capture on the PEG-gel features, as well as using a syringe pump to control the cell flow over the features. Employing fluorescent antibodies will prove helpful for both quantifying antibody presence on the PEG-gel features and detecting of the smaller 25 mm features.

Once successful PEG-gel lithography onto PDMS and single cell capture is a reality, the sensing with LSPR AuNRs can be implemented for completion of this device.

Acknowledgments:

I thank my PI, Dr. Katsuo Kurabayashi, my mentor, Dr. Robert Nidetz, the entire Kurabayashi lab for help and support, as well as the LNF staff, and the NNIN REU Program and the NSF (Grant #ECCS-0335765) for funding.

References:

[1] Lee, L. M., and Liu, A. P., J. Nanotechnol. Eng. Med. 5, 0408011-0408016 (2014).

[2] Chen, P., et al. ACS Nano 9, 4173-4181 (2015).