Microfluidic Pipette Array for Single Cell Mechanics Studies

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Introduction:

Pathological processes are mediated by a disruption in biochemical signaling, where cell mechanics can play a critical role [1]. For example, the stiffness of a cancer cell can affect its ability to deform and squeeze through the extracellular matrix to lymphatic or blood vessels during metastasis to distant organs. The study of cell mechanics requires micro-engineered tools to apply a localized force and make precise physical measurements. Micropipette aspiration is a classical tool used for mechanical analysis of single cells, and several cell mechanical properties can be measured by studying cell deformation [2]. This technique, however, is limited by its low throughput and requires highly specialized training in practice. Recently, our lab has developed a microfluidic device based on multilayer polydimethylsiloxane (PDMS) soft-lithography to study single cell mechanics in an automatic and parallel manner [3]. Through the manipulation of volume flow rates, this device is able to trap and apply a precise pressure difference across single cells. In this work, we incorporated a flow control layer intended to improve the trapping efficiency of single cells and conducted simulations and experimental studies to characterize the device. This novel microfluidic device was used to characterize the mechanical properties of human breast cells. The potential to increase the throughput of this microfluidic pipette array device will enable clinical applications in mechanophenotyping.

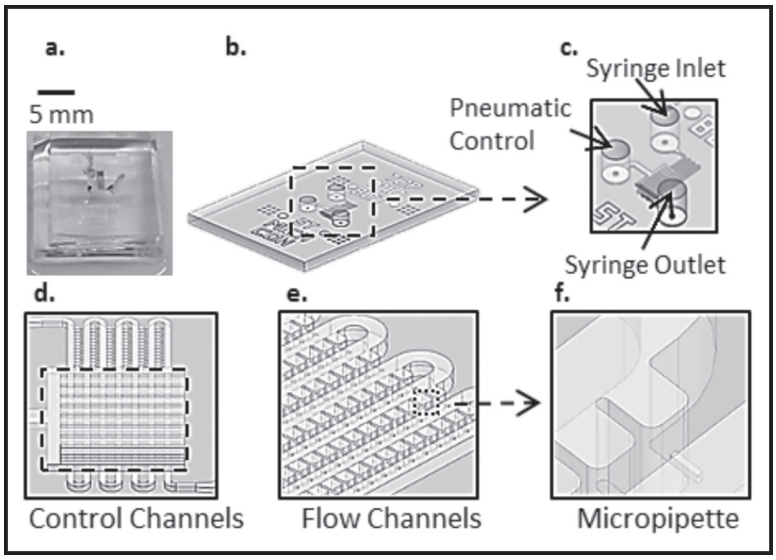


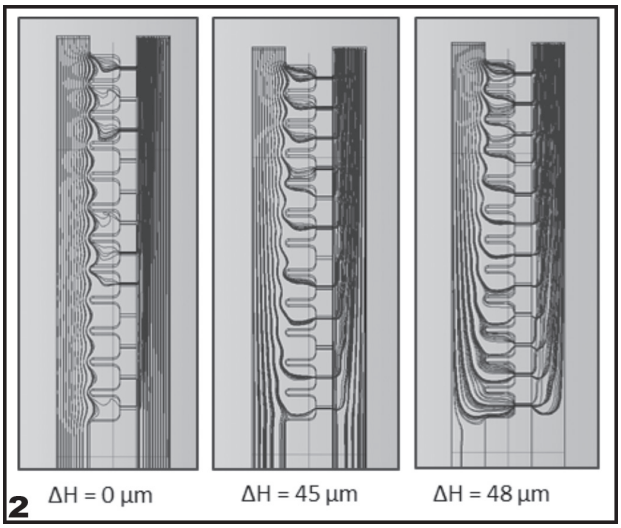
Figure 1: The microfluidic pipette array device. (a) PDMS device, (b) overall schematic, (c) device components and controls (d) control channels, (e) flow channels, (f) aspiration chamber and micropipette.

Method:

Figure 1 depicts the three main components that constitute the microfluidic pipette array. The PDMS membrane of the control channels was deflected using a pneumatic system to increase the flow resistance in the main channel and force fluid to flow through the micropipettes to load single cells into the aspiration chambers. Upon the release of the control layer, increasing the flow rate in the main channels exerted a pressure across the cell to aspirate it into the micropipette.

Three silicon molds were fabricated for PDMS casting of both flow and control layers using SU-8 photoresist patterning. The first mold defines the top half of the flow layer and micropipettes, while a second mold defines the bottom half of the flow layer. The third mold defines the control channels. After demolding the control layer, it was bonded to the top flow layer using a custom PDMS alignment setup. The top and bottom flow layers were then demolded, aligned, and bonded together to form the flow channel.

Numerical simulations of an incompressible, laminar flow model in COMSOL were used to characterize the deflection of the control channels under pneumatic pressure and the effect on single cell trapping efficiency. For experimental operation, a syringe pump was used to control the flow of cells into the microfluidic channels, and a pneumatic control was used to pressurize the control channels. An optical microscope (Nikon TiS) with 20× magnification objective was used for time lapse imaging of cell deformation. MatLab image analysis of the radius of the cell and protrusion length during aspiration can



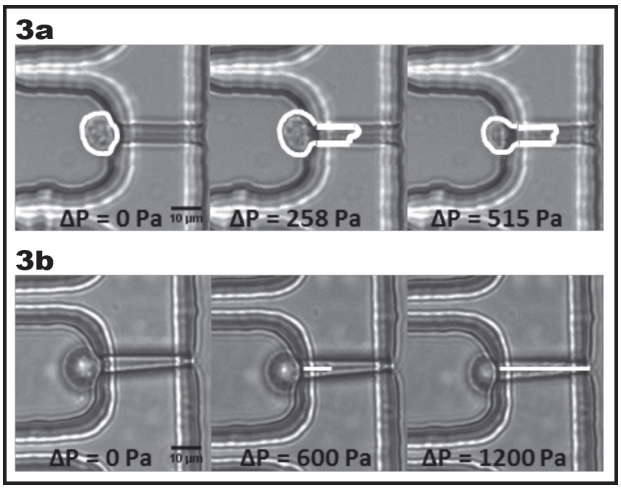


Figure 2, top: Streamlines in the microfluidic pipette array indicating where cells will follow the streamlines and be trapped in the aspiration chambers for varying deflections of the PDMS membrane, where ΔH is the deflection. (COMSOL simulations.) Figure 3, bottom: Aspiration of cells into the micropipettes. (a) MDA-MB-231 cell in a straight pipette, where the white line indicates the contour of the cell, (b) MCF-10A cell in a tapered pipette, where the white line indicates the protrusion length.

be used to determine the Young's modulus and cortical tension of human breast cell lines of non-tumorigenic MCF-10A cells and metastatic MDA-MB-231 cells.

Results and Conclusions:

Figure 2 shows the numerical simulation results of streamlines in the microfluidic channels for varying deflection of the PDMS membrane. An increase in deflection corresponds to an increase in the ratio of fluid flowing through the pipettes compared to the main channel. A deflection of 45 μm in the 50 μm channel resulted in a flow ratio greater than one, which means that more fluid is flowing through each pipette than through the main channel. Experimentally, we observed that the control channels had a minimal effect on the streamlines, and saw no significant improvements in trapping efficiency. This result is possibly due to the challenge of blocking a rectangular channel by the deflection of a PDMS membrane without rupturing the membrane or causing air bubbles to enter the flow channels through the porous PDMS. Mineral oil could potentially be used to prevent air bubbles, and using a thinner PDMS membrane and a wider flow channel might improve deflection and the flow resistance. Upon trapping, micropipette aspiration was conducted by increasing the flow rate to exert a pressure on the cells as shown in Figure 3. The lengths of the protrusions were measured in order to calculate the stiffness of the cell.

Figure 4 shows the increase in the protrusion in the micropipette of an MDA-MB-231 human breast cancer cell as the pressure difference across the cell increases over time. The Young's modulus of the MDA-MB-231 cell was measured to be 125 Pa, and the cortical tension of the MCF-10A cell is 1.47 mN/m. These preliminary results indicate that successful operation of the control channels will improve trapping efficiency to increase throughput for studying single cell mechanics. The stiffness of cells can easily be measured using the microfluidic pipette array.

Acknowledgements:

I would like to thank Professor Allen Liu, Dr. Lap Man Lee, the members of the Liu lab, and the staff of the Lurie Nanofabrication Facility for their guidance and support. This work was supported by the NSF under Grant No. ECCS-0335765 and by the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program.

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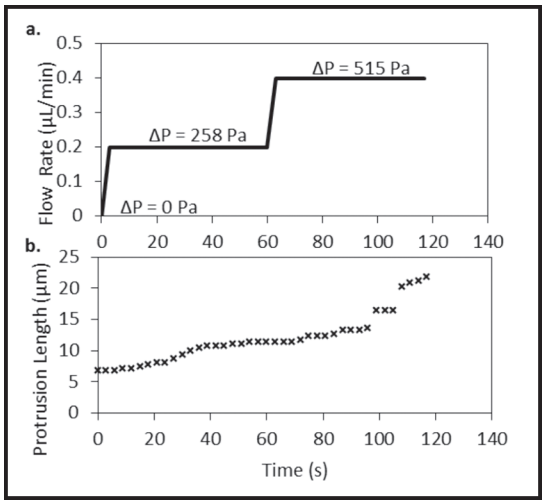

Figure 4: (a) Increasing the flow rate over time corresponds to an increasing pressure difference across the cell. (b) The pressure increases over time with a corresponding increase in protrusion into the micropipette for an MDA-MB-231 cell.