Hydrodynamically Confined Microfluidics for Single Cell Measurements

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ABSTRACT

Traditional channel-based microfluidic devices have been widely used to interrogate single cells. The ability to precisely control fluid flow and chemistry in the region of an individual cell in a microscale device has enabled a range of unique measurements. The closed nature of channel-based devices, however, limits their use in certain applications. Here, we describe a microfluidic probe that is designed to apply controlled hydrodynamic loads to cells in open liquid environments, such as Petri dishes. The probe creates a local microfluidic environment beneath the probe by flowing fluid in through one port and removing it through a second port. A device that has port geometries and flow conditions optimized for cell mechanics tests is described in this work. The device was fabricated, tested to validate performance, and applied to the measurement of cell adhesion strength.

Keywords: microfluidics, hydrodynamic confinement, cell mechanics, cell adhesion

1 INTRODUCTION

Microfluidic devices have proven to be useful tools in cell biology as they allow for precise control of chemistry and hydrodynamic loads at the scale of single cells. However, the geometry and closed nature of channel-based microfluidics can be difficult to employ for some single-cell measurements. For example, culturing certain types of cells in small closed channels is difficult and there is often limited ability to select specific cells of interest within a channel. In this work, we report on the design, fabrication, and use of a hydrodynamically confined microfluidic probe that allows a local microfluidic flow to be created in an open fluid environment such as a Petri dish or multi-well plate (Fig. 1). Probes based on hydrodynamic confined microflows (HCMs) were first reported in 2005 [1]. Since, the initial demonstration, HCM devices have been used in a range of applications, including protein patterning and local chemical treatment of biological cells (e.g., [1, 2]). Our group has focused on understanding the fluid mechanics of HCM devices [3] and developing HCM devices for probing the mechanics of biological cells. Here, we report on the design of HCM devices for applying well-controlled hydrodynamic loads in order to probe the mechanical properties of single cells.

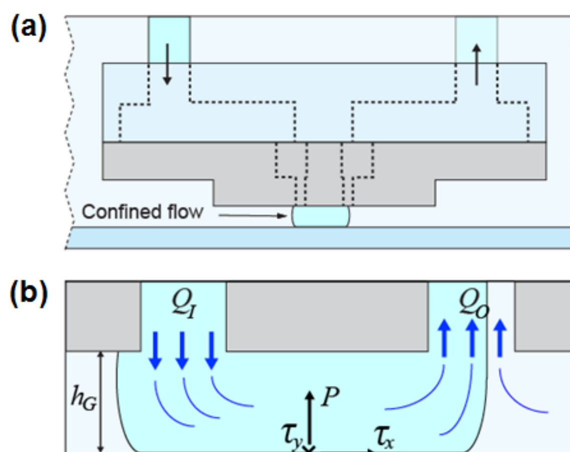

Fig. 1 – Schematic of a hydrodynamically confined microflow (HCM) probe. (a) Side view of device positioned above the bottom of liquid-filled well. The ports are in a mesa that confines high shear stresses to the region below the mesa. (b) Detail of region beneath the mesa; inlet and outlet flows create a microfluidic flow beneath the device that apply a pressure and shear stress to the bottom surface.

The mechanical properties of single biological cells can serve as an inherent marker of the condition of cell. The cell deformability and adhesion strength can be altered by the environment, cell state, and disease. For example, comparisons of healthy and cancer cells in previous work [4, 5], have shown that the cancer cells exhibit a lower stiffness and increased adhesion compared to the healthy cells. There are numerous approaches that have been demonstrated for probing the mechanics of single cells, including, AFM, optical tweezers, magnetic techniques, and hydrodynamic approaches [6]. Hydrodynamic loading, in

which shear and pressure loads induced by fluid flow are applied to the cell, have been used for decades in cell mechanics measurements [7]. Traditionally, such tests were performed in parallel-plate flow chambers. However, more recently, microfluidic channels have been used in cell mechanics measurements. The principle advantage of generating hydrodynamic loads in small-scale channels is that the small dimension of the channels allows higher shear stresses to be achieved in the laminar regime [8], thus enabling higher strength and stiffness cells to be tested.

The objective of this work was to develop a HCM device to apply uniform shear stress to a single adhered cell in an open liquid environment. The design, fabrication, and testing of the device is briefly summarized here.

2 DEVICE DESIGN

A device to produce an HCM requires at least two ports: an inlet and an outlet. The inlet and outlet flow rates at the two ports are controlled by syringe pumps. The shape of the flow envelope beneath the device and the hydrodynamic loads on the surface beneath the device are determined by the flow rates and port geometry. Previous devices have primarily used simple circular, square, or rectangular ports [3]. Here, we explored a broader range of port geometries in order to understand how port size, spacing, and shape could be optimized to generate uniform shear stress on cells positioned beneath the device. The port geometry was set to generate a uniform shear stress region beneath the device that is at least 150 μm x 150 μm with less than 1% variation in shear stress over the region. Computational fluid dynamics (CFD) simulations were performed to examine different designs and assess the flow characteristics. Fig. 2 shows the optimized port geometry and shear stress on the surface beneath the device for a specific flow-rate. This port geometry allows for similar shear stress uniformities within the marked region over a range of flow rates that produce shear stresses with relevant magnitudes for cell mechanics tests.

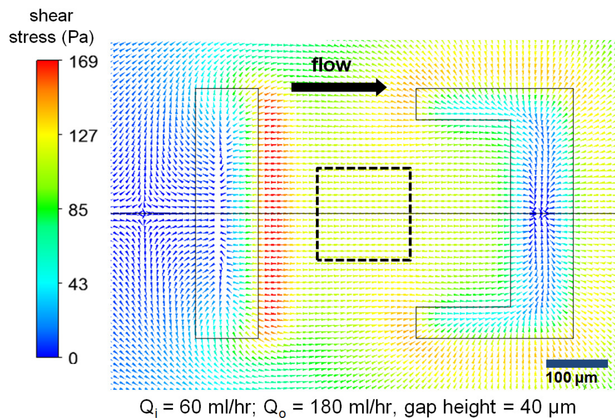

Fig. 2 – CFD prediction of shear stresses on surface beneath device for optimized port geometry. The shapes of the optimized ports are shown (inlet-left, outlet-right). The dashed square indicates the uniform stress region.

3 DEVICE FABRICATION

The HCM devices are fabricated using bulk silicon micromachining. A picture of the port side of the device is shown in Fig. 3. The ports are etched in a 3 mm x 3 mm mesa, as seen in Fig. 3. The backside of the device consists of larger silicon ports that facilitate connections to the inlet and outlet tubing. The silicon device is fabricated using a combination of photolithography and deep reactive ion etching. Briefly, thermal oxide is grown on both sides of a 500 μm thick double-side polished Si wafer, photolithography and a wet etch are then used to pattern the oxide on the back side of the wafer, and then channels (300 μm deep) are etched using deep reactive ion etching (DRIE). Next, the mesa and ports are patterned on the front surface and etched to a depth of 200 μm using DRIE. The remaining thermal oxide on the front and back surfaces of the wafer are then removed and the devices are segmented using a dicing saw. Fluid connections are made to the backside of the device by plasma bonding a molded PDMS layer to the Si device and then inserting tubing into punched holes in the PDMS.

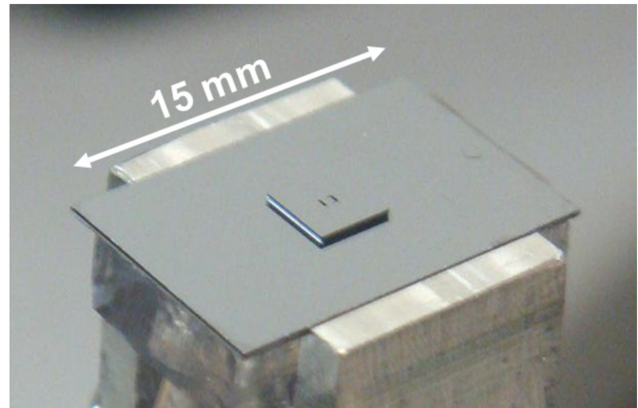

Fig. 3 – Photograph of port side of HCM device. The ports positioned in the center of mesa are visible in this picture.

4 EXPERIMENTAL METHOD

The HCM devices, fabricated as described above, are mounted into a machined holder that is fixed to a 5-axis translation and alignment stage. The stage consists of a commercial 3-axis translation stage and a custom 2-axis tip-tilt stage. The stage with the device is mounted on an inverted microscope such that it can be positioned in a dish containing cells while simultaneously viewing the cells with bright field or fluorescent microcopy from below. The tubes from the inlet ant outlet are connected to standard syringe pumps. The pressure between the inlet and outlet ports is measured using a commercial MEMS pressure

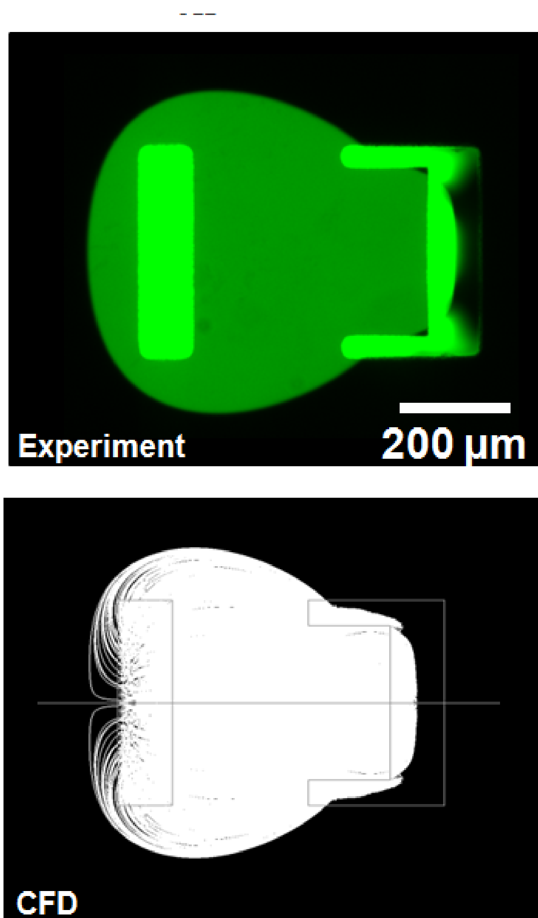

Fig. 4 – Comparison of flow envelope measured in experiments (top) and predicted by CFD (bottom). Results are shown for an inlet and outlet flow rate that produces a shear stress of 38 Pa in the uniform stress region.

sensor (Honeywell, #26pcafa6d). The pressure between the ports is measured as it provides a feedback measurement to assess the separation of the device from the bottom of the dish. The basic experimental setup and procedures used here are similar to those reported in ref. [3].

In the cell experiments reported here, 3T3 fibroblasts were cultured on patterned collagen patches of various size and shape. The islands were patterned via micro-contact printing and were sufficiently small that a single cell filled the entire island. The shapes and sizes of the islands were systematically varied to examine the effect of cell geometry on cell adhesion strength.

A typical cell adhesion test is performed by positioning the device above the cell, adjusting the tip/tilt and device height so the device is parallel and separated from the bottom of the dish by 40 µm. Over the course of a test, the shear stress applied on the cell is systematically increased by changing the flow rate. Specifically, a stepped flow application is used in which the stress is increased in increments of 4.7 Pa. After each increase in stress, the stress is held constant for 8 seconds. The flow rate (and hence shear stress) is increased until the cell detaches from the substrate. The stress applied at detachment is considered the adhesion strength of the cell.

5 RESULTS

Detailed results are not presented in this short conference paper due to limited space. Rather, example results that demonstrate the performance of the device are included here. Complete and detailed results will be presented at the conference and in subsequent papers.

The flow behavior of the device was characterized in a series of two studies. First, a fluorescent solution was flowed through the device and the flow envelope beneath the device was imaged (Fig. 4). The measured shapes of the flow envelopes were in good agreement with the CFD predictions over a range of flow rates. Second, the hydrodynamic loads applied on the surface were characterized by generating an HCM above a substrate composed of a soft gel with fluorescent beads adhered to the surface. The displacements of the beads were tracked using microscopy to assess the deformation of the surface and compared to predictions. Again, the experiments validated the CFD-predicted device performance.

Cell adhesion tests were performed on patterned fibroblasts, as described in section 4. A sequence of images from a test on a patterned cell is in shown in Fig. 5. This cell is cultured on a square island and the outer boundary of the cell matches the shape of the island, the nucleus is visible in the center of the cell. The first image is prior to loading, the second is at an applied shear of 38 Pa, and the final is at a shear of 80 Pa, which is immediately prior to detachment for this cell. This basic sequence demonstrates how a cell deforms during detachment. Adhesion tests across various shape and size islands were performed to probe the role of cell geometry in adhesion strength.

Fig. 5 – Sequence of images from a cell adhesion test on single patterned fibroblast. The flow direction is from the bottom of the image to the top of the image. The right image is of the cell under zero stress, the middle image is at 38 Pa, and the right image is at 80 Pa, immediately prior to detachment.

6 CONCLUSIONS

We have developed and demonstrated an HCM device for single-cell mechanical tests. The device allows uniform shear stresses to be applied to single-cells that are cultured in open liquid environments. The port geometry of the device has been optimized to produce a uniform shear stress over a region that is larger than a typical single cell. The device was fabricated using silicon micromachining and the performance was validated using flow tests and measurements of surface tractions. Finally, the device has been applied to characterize the adhesion strength of patterned 3T3 fibroblasts. This device provides a flexible platform for applying well-controlled hydrodynamic loads to single biological cells.

REFERENCES

[1] D. Juncker, H. Schmid, and E. Delamarche, "Multipurpose microfluidic probe," *Nature Materials,* vol. 4, pp. 622-628, Aug 2005.

[2] M. A. Qasaimeh, S. G. Ricoult, and D. Juncker, "Microfluidic probes for use in life sciences and medicine," *Lab on a Chip,* vol. 13, pp. 40-50, 2013.

[3] K. V. Christ and K. T. Turner, "Design of hydrodynamically confined microfluidics: controlling flow envelope and pressure," *Lab on a Chip,* vol. 11, pp. 1491-1501, 2011.

[4] J. Guck, S. Schinkinger, B. Lincoln, F. Wottawah, S. Ebert, M. Romeyke, D. Lenz, H. M. Erickson, R. Ananthakrishnan, D. Mitchell, J. Kas, S. Ulvick, and C. Bilby, "Optical deformability as an inherent cell marker for testing malignant transformation and metastatic competence," *Biophysical Journal,* vol. 88, pp. 3689-3698, May 2005.

[5] S. P. Wankhede, Z. Q. Du, J. M. Berg, M. W. Vaughn, T. Dallas, K. H. Cheng, and L. Gollahon, "Cell detachment model for an antibody-based microfluidic cancer screening system," *Biotechnology Progress,* vol. 22, pp. 1426-1433, Oct 2006.

[6] K. J. Van Vliet, G. Bao, and S. Suresh, "The biomechanics toolbox: experimental approaches for living cells and biomolecules," *Acta Materialia,* vol. 51, pp. 5881-5905, Nov 2003.

[7] K. V. Christ and K. T. Turner, "Methods to Measure the Strength of Cell Adhesion to Substrates," *Journal of Adhesion Science and Technology,* vol. 24, pp. 2027-2058, 2010.

[8] K. V. Christ, K. B. Williamson, K. S. Masters, and K. T. Turner, "Measurement of single-cell adhesion strength using a microfluidic assay," *Biomedical Microdevices,* vol. 12, pp. 443-455, Jun 2010.