Microfluidic Chip to Measure Response of Single Cells to Extracellular Stimuli

CNF Project # 990-01
Principal Investigator(s): Susan Z. Hua
User(s): Tom Pennell

Affiliation(s): Dept. of Mechanical & Aerospace Engineering, SUNY Buffalo
Primary Research Funding: NSF
Contact: zhua@eng.buffalo.edu, tpennell@eng.buffalo.edu

Abstract:
We have developed a microfluidic chip that is capable of measuring volume change in single cells in real-time. The sensor chip was constructed using photosensitive polymer (SU-8) on a Pyrex® glass substrate. Using MDCK cells, we have measured the response of a single cell to osmotic challenges. The results show that the impedance based sensing provides the required sensitivity and resolution to detect volume change down to a single cell. The microfluidic chip allows rapid and convenient change of solutions, enabling detailed studies of various drugs and chemicals that may play important role in cell physiology at the single cell level.

Summary:
The transport of ions and other solutes across a cell membrane reflects cellular metabolism, neurotransmitter activity, as well as interaction with extracellular environment [1,2]. Since transport activities introduce the changes in intracellular and extracellular osmolarity, it causes the cells to change their volume. Thus, real time measurements of cell volume provide direct access to studying transport activities and cell physiology. The microfluidic lab chip enables handling and analysis of single cells on chip in a convenient manner, and provides continuous delivery of various solutions with precise control. It is an ideal platform for studying cellular physiology at the single cell level [3].

We have developed a lab-chip that can measure the volume changes of a single cell in real-time. We demonstrate that the impedance based sensing method can be utilized to measure the activities of single cells with high sensitivity and resolution. The chip allows rapid change of solutions enabling high throughput screening of cell response to extracellular stimuli. Moreover, the morphological change of cells can be simultaneously monitored using an optical microscope.

The sensor chip exploits the fact that cell membranes are electrical insulators at low frequencies. As in a coulter counter where the cells passing through an orifice change the impedance of the aperture, a captured single cell in a defined sensing zone of the chip causes the resistance of the sensing zone to change [4,5]. In this work, a narrow sensing region was created along the fluidic pathway, within which a chevron like feature was designed to capture suspended cells in the solution. Once captured, various solutions can be readily perfused through the channel, and the resulting
changes in cell volume can be followed via impedance measurements in real-time. The single cell sensor chip was constructed using photosensitive polymer (SU-8) on a Pyrex glass substrate. A standard optical lithography technique was used to fabricate the sensor chip. Using the lift-off technique, 200 nm thick thin film platinum electrodes were deposited on the glass wafer by e-beam. The fluidic channel was then constructed using SU-8 photoresist following the standard processing protocol. A programmed temperature ramp-up hard-baking process was applied after lithography; this process also helped to heal the micro cracks in the SU-8 structure.

The single cell sensor was tested by measuring the impedance change due to the capture of individual single-cell. The cell capturing events in the microfluidic channel were simultaneously monitored using an optical microscope. A dilute cell suspension of MDCK cells in isotonic solution was then perfused through the channel inlet at a flow rate of 1.02 µL/min giving a linear velocity of approximately 1 mm/s between the chevron. A stepwise increase was observed whenever a cell was captured.

The typical sensor output of a single cell capturing event was 8 to 14 mV, depending on the original size of individual cells. The cell volume change in response to changes in extracellular solution was recorded as a function of time. When a single cell was arrested in the sensing zone, the solution was switched to isotonic solution (without cells) to establish a baseline representing the resting cell volume. The channel was then perfused with hypotonic solution to cause cell swelling. The volume change of the single cell in response to hypotonic challenge was measured in real-time using impedance measurement, and the change in cell volume was simultaneously recorded using optical microscopy. Results show that both maximum swelling and the time course of the swelling are different in different cells.

References:


Figure 2: Optical images of a suspended single cell in an isotonic solution (A) and hypotonic solution (B), respectively. The cell is seen captured between a V-shaped feature inside the microfluidic channel.

Figure 3: Real-time cell volume change when the solution in the channel is changed sequentially from isotonic (326 mOsm) to hypotonic solution (187 mOsm), then back to isotonic solution (A). Sensor output without cells in the channel when the solutions was perfused following the same protocol as in (B).