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Microfluidic Cell Volume Biosensor for High Throughput Drug Screening

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ABSTRACT

The maintenance of cell volume is critical to health. Cell volume change reflects many biological and physiological processes. We have developed a lab-chip to measure cell volume change in real-time with high sensitivity and resolution, and applicable to both adherent and suspended cell populations. The volume change was detected by measuring the impedance of extra-cellular solution within a microfluidic chamber containing the cells. Using microfabrication to make precise chamber dimensions, volume change can be detected in response to an osmotic gradient <1mOsm. The sensor provides rapid screening of pharmaceutical agents affecting cell volume. We have screened for peptides that affect cell volume regulation and found one in spider venom that inhibits at ~100pM.

INTRODUCTION

A cell's volume is an integrated function of its physiological activity [1]. Changes of cell volume accompany growth and death as well as reflecting more subtle homeostatic changes[2,3]. A simple real time monitor of cell volume alteration can provide insight into many different aspects of cell life including excitability, metabolism, apoptosis, necrosis, neurotransmitter responses, and environmental toxicity. It is possible to use the kinetic response of cell volume as an indicator to achieve a cell based screen. There are a variety of methods to measure cell volume, which include confocal microscopy, electron microscopy [4,5], laser scattering, fluorescence intensity measurement [6,7], Coulter counter [8], and other electrical impedance measurements [9]. However, most methods to measure cell volume are time consuming, and either lack-resolution or require complex apparatus. They also cannot provide real-time measurements. Electron microscopy suffers from the need for fixation that can have a profound effect on cell volume. While a relatively rapid method, the Coulter counter requires free-floating cells, where isolation itself can produce significant physiological changes.

We have developed a microfluidic lab-chip biosensor that utilizes cell volume changes to monitor how cells respond to drugs or toxic chemicals. The biosensor is based on an electrical impedance method to measure changes in cell volume. In this method, adherent cells on a solid substrate were placed in a shallow microfabricated chamber. As the volume of the cells change, they displace the extra-cellular fluid in the chamber, thereby changing the electrical resistance of the chamber. Taking advantage of microfabrication, the dimensions of sensing chamber can be precisely controlled that results in high sensor resolution and sensitivity. This method is non-invasive, and it provides real-time measurement of changes in cell volume for both adherent and suspended cells. The microfluidic nature of the device also offers a parallel processing platform to enable high throughput screens for drug discovery.

Using this sensor, we tested volume changes of cultured primary astrocyte cells in response to various osmotic pressures. We observed regulatory volume decrease (RVD) when the cells were exposed to hypotonic solution, and regulatory volume increase (RVI) when the cells were exposed to hypertonic solution. We further screened for peptides that affect cell volume regulation and we found one in spider venom that inhibits at ~100pM. The results show that the sensor is simple to fabricate, it is robust and reliable after hundreds of experiments. While our prototype contained a single fluid channel, the sensor chip can be easily extended to multichannels for high throughput screening.

EXPERIMENTAL DETAILS

A prototype microfluidic cell volume sensor was built on silicon as shown in Figure 1. The sensor is configured with a single fluid channel connected with inlet and outlet reservoirs. Two sensing chambers 1.5mm wide and 3.75mm long (labeled Chamber I and II in Fig. 1) were defined along the fluidic channel. Chamber-I is designed as the cell-testing chamber with a depth of 15 μm. Chamber-II is deeper (55 μm), it serves as the calibrating chamber for monitoring solution resistivity (Fig. 1). Four platinum electrodes are fabricated in each chamber to form a four-point probe for impedance measurements.

High resistivity silicon wafers were used as the substrate, and multiple steps of KOH etching were performed to fabricate the channel and chambers. Following the etching process, 1 μm of silicon oxide layer was deposited on the top of wafer using plasma enhanced chemical vapor deposition (PECVD) minimize electrical interaction between the substrate and solution. Platinum electrodes, 250 nm thick, were then deposited by e-beam deposition using the lift-off technique. Another 1 μm thick layer of PECVD silicon oxide was deposited on top of the fabricated features except for the sensing and contact regions. To reduce electrode impedance and improve the signal to noise ratio (SNR) we plated electrode surfaces with platinum black. The micro-porous structure of platinum black increases the active surface area of electrodes and provides better ion exchange. The fabricated sensor chip was then glued to an acrylic platform (not shown). The acrylic platform contains a three way fluid input connection, which aligns with the inlet reservoir of the chip in Fig.1 from the backside (for changing testing solutions), and also provides a fluid outlet line connected with the outlet reservoir of the chip.

For testing, the cells were cultured on normal glass cover slips, and placed on the top of the sensor chip with adherent cells facing the chambers. The coverslip was pressed against the chip with a clamp that applied uniform pressure of ~50N. For testing, an active current source provided a 1 μA, 50 Hz sinusoidal signal to the two outer electrodes. The chamber resistance was measured using a home built JFET instrumentation amplifier (providing low input current to reduce electrode polarization) and a lock-in amplifier.

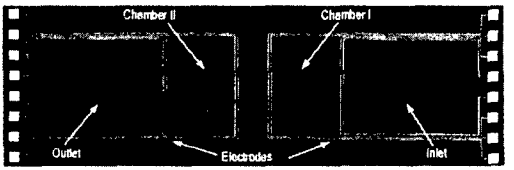

Figure 1. Optical micrograph of sensor made in silicon. There are two measuring chambers in series, 1.5mm x 3.75 mm.

RESULTS AND DISCUSSION

Cell volume response to anisotonic stimuli

To characterize the sensor, we measured the volume change of tissue cultured astrocyte cells using anisotonic stimuli. The primary rat astrocytes were grown on a glass cover slip under standard culture conditions. The osmolarity was adjusted using mannitol, so that the ionic strength was unaffected. Prior to each experiment, solution conductivity was checked using a conductivity meter, and solutions were titrated with NaCl to equal conductivity. The flow rate was maintained at ~0.3 µl/s.

The cells were initially perfused with isotonic solution (323 mOsm), followed by anisotonic solution, causing cell swelling/shrinking. The solution was then switched back to isotonic upon volume regulation completion. Figure 2a shows the astrocytes response to perfusion with hypotonic solution (188 mOsm). As shown in fig. 2a there is a rapid increase in volume as a result of water influx, followed by a slow decrease in volume which is rate limited by the loss of osmolytes to the extracellular solution — the well known regulatory volume decrease (RVD)[10]. It is known that the RVD mechanism of astrocytes are due to K^+ and Cl^- efflux through individual ion channels as well as the release of organic osmolytes, such as taurine [11]. Upon returning to isotonic solution at the end of RVD we observed cell volume shrinkage due to water activity only. Since the intracellular osmolarity was balanced with extracellular osmolarity at the end of RVD, perfusing cells with isotonic solution elevated the extracellular osmolarity and resulted in this volume shrinkage. We did not observe the secondary RVI, which is expected for many cell types.

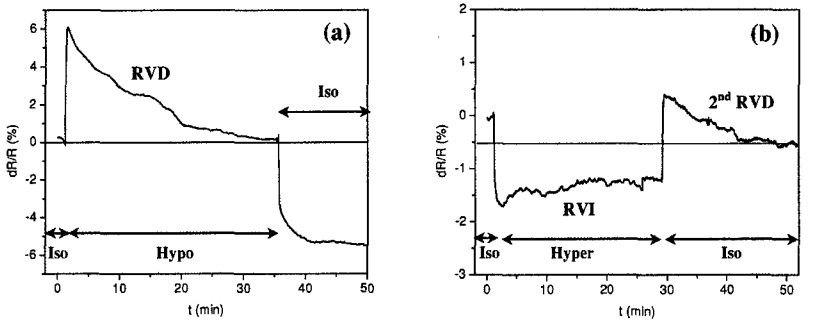

Figure 2. Response of astrocytes due to osmotic stimuli. (a) Volume changes of astrocytes due to hypotonic stimuli. The chamber was first filled with isotonic solution (321 mOsm) until the system stabilized, then perfused with hypotonic solution (188 mOsm) to observe swelling and RVD, followed by a return to isotonic solution at the end of RVD. (b) Volume changes of astrocytes due to hypertonic stimuli (345 mOsm). The chamber was initially perfused with isotonic solution, then hypertonic solution (345 mOsm), finally switched back to isotonic solution. The secondary RVD is shown.

Figure 2b shows the astrocytes response to perfusion with hypertonic stimulus (345 mOsm). Hypertonic solutions caused rapid shrinking of the cells followed by a regulatory volume increase (RVI), as shown in Fig. 2b. The RVI mechanisms of astrocytes are also recognized as influx of Na^+, K^+, and $2Cl^-$ by co-transporters and uptake of organic osmolyte through Na^+ concentration regulated channels [12]. Upon switching back to isotonic solution after RVI, we observed a significant secondary RVD (see fig. 2b). Similar results have been previously reported for astrocytes [9,13].

Chamber resolution testing

The resolution of the lab chip is defined by its ability to detect miniscule cell volume changes in response to minute osmotic gradients. We have determined the resolution of the chip to measure the cell response to an osmotic gradient as small as 1 mOsm. Using tissue cultured primary astrocytes, the chamber was first perfused with isotonic solution (321 mOsm) for a few minutes, and then switched to hypotonic solution (311 mOsm) corresponding to a low osmotic stimulus of 10 mOsm, as shown in Fig. 3a. As shown in Fig. 3a, once the cell swelling peaked, we switched the perfusate back to the isotonic solution. Cell swelling could be detected even when the osmotic stimulus from the solution differed by only 1 mOsm, as shown in Fig. 3b.

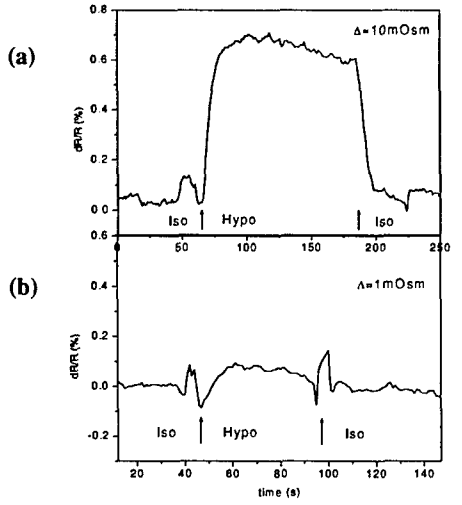

Figure 3. (**a**) The response of astrocytes to a drop of 10 mOsm in extracellular solution. (**b**) The response to a drop of 1 mOsm in extracellular solution. The vertical arrows indicate a change of solution.

Screening of pharmaceutical agents

The biosensor was developed to allow rapid screening of reagents only available in small quantities. To test its performance as a pharmacological screening device we screened peptides isolated from the tarantula *Grammostola spatulata* [14]. The control test was conducted using anisotonic stimulus without peptide. In Fig. 4, the dotted black curve shows the RVD of astrocytes subjected to a 188 mOsm hypotonic stimulus. Varying concentrations of the peptide, called GsMTx1, from 1μM to 100 pM were added to the hypotonic solution, each time a fresh culture was used. RVD was fully blocked at 1μM, 10nM, and 1nM, as shown in Fig. 4A, and reduced to about half the normal rate at 100 pM. This apparent high affinity suggests that GsMTx1 is specific for a key component of RVD. Following each test, we perfused the chamber with isotonic media for 10 minutes to wash out the peptide, and then challenged the cells with hypertonic media (188 mOsm) without peptide. We have observed the cell swelling due to water activities across the cell membrane; however, the RVD was not recovered (Fig. 4B). The results demonstrate the capability of the volume sensor as a sensitive probe for toxin detection.

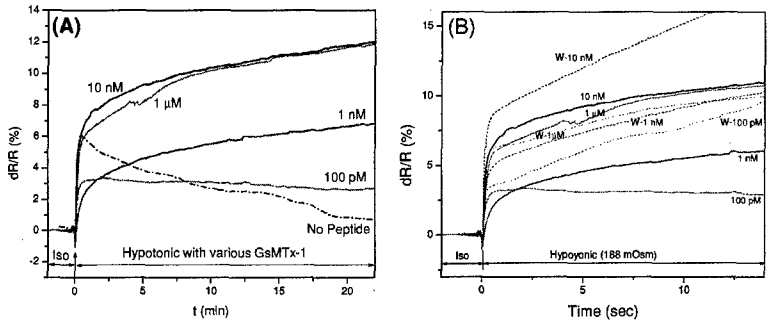

Figure 4. **(A)** Volume regulation behavior of astrocytes challenged with 188 mOsm saline in the presence of GsMTx1. Fresh cultures were used for each concentration. GsMTx1 at 100PM eliminates about half of the volume regulation capability. (B) Volume response to hypotonic media (dashed lines) after the peptide was washed in isotonic media for 10 minutes. The volume responses to peptide, the same as in (A), were also plotted for comparison (solid lines). Curves with prefix 'W' stand for measurement after washing.

The chamber resistance with and without cells has been tested in isotonic solution for extended periods of time. In both cases, the resistance could be kept constant for hours with variation in resistance less than 15 mV (less than 0.5% of total resistance). Also the volume sensor has been repeatedly used for several hundreds times and showed consistent results.

CONCLUSION

This volume sensor was made in silicon for fabrication convenience, but the technology can be readily transferred to plastic, where it would be possible to create thousands of inexpensive parallel channels in a small device. The fluidic and electronic technology is elementary and robust, so that sensor arrays can be made battery powered and disposable. The ability to rapidly scan a variety of cell types against different pharmacologic agents permits high throughput drug screening.

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