STUDY OF SINGLE CELL KINASE ACTIVITY USING AN AUTOMATED MICROFLUIDIC DEVICE

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ABSTRACT

Kinase enzymes are prevalent within cells and are responsible for regulating cellular functions, yet little is known about how these enzymes function in signaling transduction pathways. Our goal is to develop an automated microfluidic device capable of analyzing in vivo kinase activity one cell at a time.

KEYWORDS: Single Cell Analysis, Kinase Enzymes

INTRODUCTION

The goal of this research is to develop a fully automated microfluidic device capable of collecting kinase activity data at the single cell level. Single cell level analysis is needed to study the potential biological importance of heterogeneous cellular response common among cell populations.

To study kinase activity, peptides specific for a kinase enzyme of interest have been developed. The current peptide being used is recognized as a substrate by protein kinase C (Figure 1). The peptide is labeled with a fluorescent tag (FAM) for detection of both the initial substrate (SPKC) and peptide product (PSPKC) resulting from enzyme modification. A myristoyl (MYR) group is attached to the peptide via a disulfide linkage. MYR is a C14 fatty acid which provides enough lipophilicity to the peptide to allow it to passively cross the cell membrane. Once the peptide enters the cell, the disulfide bond is cleaved in the reducing environment of the cytosol, removing the MYR group. The remaining peptide is recognized by the enzyme and phosphorylated according to the enzyme activity.

5-FAM-Arg-Phe-Ala-Arg-Lys-Gly-Ser-Leu-Arg-Gln-Lys-Asn-Val-**Cys***-[s-s-Lys(MYR)-Lys-Lys-Lys-NH2]

Figure 1. A synthetic peptide substrate for protein kinase C (MYR-ss-SPKC). The peptide is labeled with fluorescein for detection and made membrane permeable through attachment of a MYR group.

The microfluidic device designed to perform single cell analysis is shown in Figure 2. Cells are hydrodynamically transported through an intersection containing an electric field. As the cells pass through the electric field, electrical lysis occurs and the cell lysate is electrokinetically injected into a channel perpendicular to the hydrodynamic flow. Subsequent electrophoretic separation and fluorescence detection of the substrate and product peptides occurs. Collection of single cell data on this channel network has been previously demonstrated using cytosolic dyes with analysis rates of up to 12 cells per minute [1].

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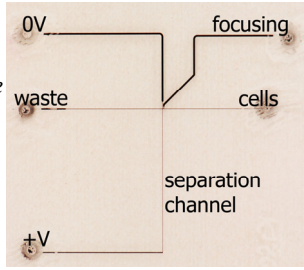
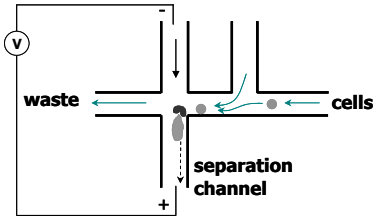
Figure 2:
a) Image of microfluidic device for single cell analysis
b) Schematic of cell transport and lysis on chip





EXPERIMENTAL

Microfluidic devices are prepared from white crown glass using traditional photolithography, etching, and bonding procedures [2]. After device preparation, the channels are coated as necessary to prevent cellular adhesion and as appropriate for the separation of the substrate and product peptides. The experimental set-up for single cell analysis is shown in Figure 3.

All experiments are performed using Jurkat cells (TIB-152). Extracellular buffer (ECB) for cell washing contains 10 mM HEPES, 5 mM KCl, 135 mM NaCl, 2 mM $MgCl_2$, 2 mM $CaCl_2$, pH 7.4. Peptide separation buffer consists of 50 mM TRIS, 10 mM Boric Acid, 1 mM Spermine, 30 mM Polyethylene Glycol (PEG), and 1 mM TCEP-HCl, pH 9.0.

For loading, cells are incubated for 15 to 30 minutes in a concentrated solution of the reporter peptide (Figure 1) diluted with ECB containing 10 mM glucose. After incubation, cells are washed 4 to 5 times with ECB to remove extracellular reporter peptide. Cell loading is confirmed through observation of cellular fluorescence. Loaded cells are then placed in the cell reservoir on the chip for analysis. All other chip reservoirs are filled with separation buffer.

Electrophoretic separation conditions for SPKC and PSPKC were developed using peptide standards (Figure 4). The separation channel is coated with PolyE323 to reverse the electroosmotic flow as well as to reduce peptide interaction with the channel wall [3].

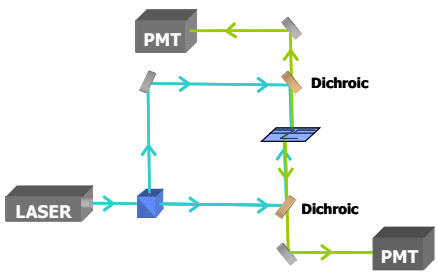


Figure 3. Experimental set-up. Two detection channels were implemented; one channel to detect the arrival of a cell at the lysis intersection and the other channel to detect the electrophoretically separated analytes

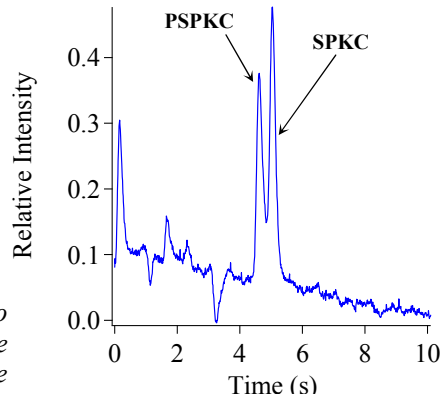


Figure 4. Electrophoretic separation of SPKC and PSPKC standard peptides

RESULTS AND DISCUSSION

An example of single cell kinase activity data collected on the device is shown in Figure 5. The cells were loaded with 250 μM MYR-ss-SPKC peptide. An overlay with the standard separation shown in Figure 4 identifies the initial two peaks of the three observed for each cell as the PSPKC and SPKC peptides, respectively.

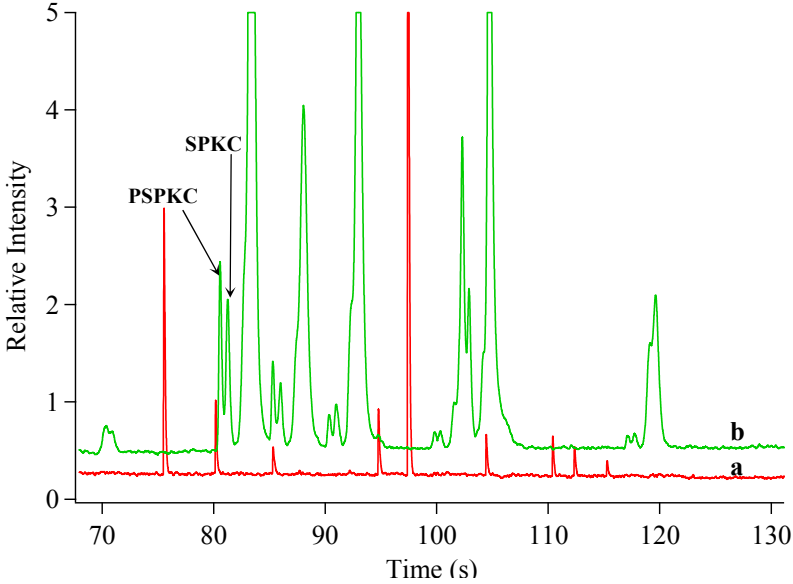


Figure 5. Example single cell kinase data demonstrating the rapid analysis of six cells. Trace **a** *displays the cytometry data to show a cell prior to lysis and provide a time "0" for the lysate analyte migration times. Trace* **b** *displays the resulting peaks from the cell lysate detected in the separation channel. The PSPKC and SPKC peptides are identified through migration comparison with a standard separation.*

CONCLUSIONS

Using this microfluidic device rapid analysis of single cell kinase activity is demonstrated. Kinase activity will be assessed by the substrate/product ratio. Research is now focused on developing coatings to increase the throughput of the device and design of on-line incubation capabilities.

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