MICROFLUIDIC DEVICE WITH INTEGRATED ANTIBODY ARRAYS FOR CELL SIGNALLING ANALYSIS

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ABSTRACT

In this paper we present the integration of protein arrays with a microfluidic device for analysis of cell signaling pathways. The device is capable of performing all the steps needed in que-signal response analysis of signaling pathways, including cell stimulus, cell lysis and protein analysis. The integration of protein arrays is realized with little additional complexity to the device fabrication process.

Keywords: Antibody arrays, Cell lysis, Cell signaling, Protein arrays

1. INTRODUCTION

Quantitative data on the dynamics of cell signaling induced by different stimuli (ques) requires large sets of self-consistent and dynamic measures of protein activities, concentrations, and states of modification. The labor intensive nature of these experiments strongly limits the amount of data available. We have previously presented a microfluidic segmented flow device that could achieve rapid stimulus and lysis of cells [1][2]. The device had an integrated thermal control set-up for optimal performance during stimulus and lysis. However, analysis of the cell lysates were performed off-chip using quantitative western blotting, limiting the throughput and automation that can be achieved with the device. By on-chip integration of protein arrays with the previously presented device a highly integrated microfluidic device for the analysis of cell signaling has been realized.

2. DESIGN AND FABRICATION

The device consists of a fluidic system defined using soft lithography in a PDMS layer bonded to a glass slide (figure 1). Protein arrays are pre defined on the glass slide along with alignment features to guide the placement of the fluidic system. The fluidic system contains two main zones, one for cell stimulus and one for cell lysis. The protein arrays are placed at the end of the lysis zone just before the outlet. During the oxygen plasma surface activation of the glass slide prior to bonding of the fluidic system, the protein arrays are protected from the plasma using small PDMS stamps. Besides alignment requirements, the integration of the protein arrays thus adds little additional fabrication complexity.

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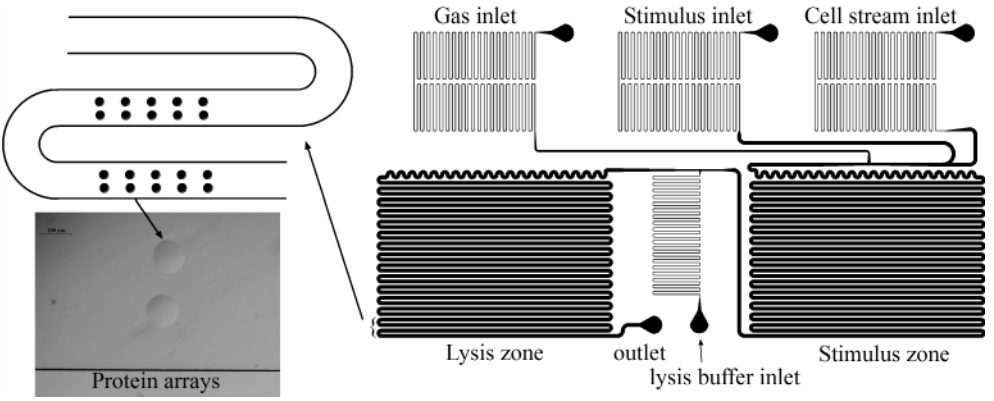

Figure 1: Schematic of the fluidic system, with two main zones, one for cell stimulus and one for cell lysis. There are three primary inlets ports, one for the cell stream, one for the stimulus and, a gas inlet for formation of the segmented gas-liquid flow. Cell stimulus takes place in the first zone and cell lysis is initiated in the second zone by introducing a lysis buffer via a secondary inlet located between the two zones. Protein arrays are placed at the end of the lysis zone. The optical image shows protein spots after bonding of fluidic system on top. The printed spot volume was 1 nl, resulting in a spot diameter of ~200μm. No damage to the spots was observed as a result of bonding of the fluidic system.

3. RESULTS AND DISCUSSION

Cell signaling analysis using western blotting has shown that the device is capable of very fast and reproducible stimulation and lysis of cells (figure 2A and 2B). However, large amounts of BSA present in the cell lysates can distort the electrophoretic separation of proteins of similar size as BSA during western blotting, making it difficult to analyze many important signaling proteins such as JNK (figure 2B) and AKT. Protein arrays alleviate this

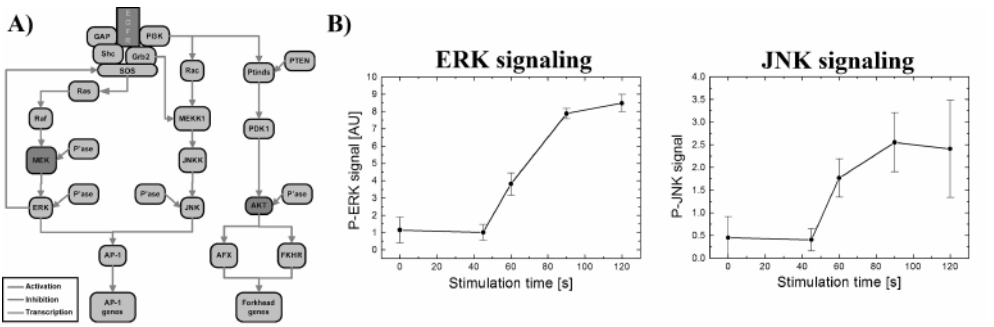

Figure 2: A) Schematic of typical cell signaling networks. B) Western blot analysis of ERK and JNK signaling in Jurkat E6-1 cells stimulated with α-CD3 for different times. The JNK signal is distorted (large error bars) due to large amount of BSA in the lysate.

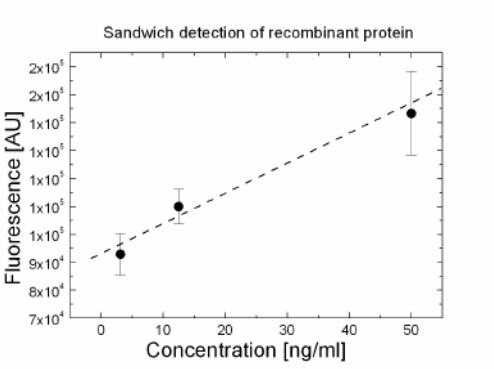

Figure 3: Sandwich detection of recombinant AKT at different concentrations using Anti-phos AKT secondary antibody and Strepavidin APC as detection reagent.

problem by being highly specific towards the capture anti-body and have previously been used to quantify protein activation in cell signaling [3]. In this study anti-AKT anti-body arrays were spotted at 1nl per spot in a PBS/glycerol solution resulting in spots of approximately 200µm diameter (figure 1). A sandwich array is used for detection, where the secondary anti-body is fluorescently labeled. To ensure that the integrity of the arrays were intact after bonding of the fluidic system and in order to estimate the performance of the arrays, a dilution series were performed (figure 3). The results show that the protection of the arrays during the bonding works and that we are capable of detecting protein concentrations below 5ng/ml. Reference spots of BSA conjugated to CY5 showed approximately 10% variation across experiments.

This work presents an integrated device for complete analysis of cell signaling which can be integrated into an automated system and thereby expands greatly the number and reliability of experiments designed to collect information on signaling pathways.

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