MICROFLUIDIC DEVICES FOR STUDYING THE RESPONSE OF ADHERENT CELLS UNDER SHORT TIME STIMULI TREATMENT

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ABSTRACT

This paper reports a microfluidic method for studying protein pathways of adherent cells under short stimulation times. The device is capable of performing all the steps needed in stimulus-signal response analysis of signalling pathways by an immunocytochemical assay (In-Cell Western), including cell culture, cell stimulus, cell fix, and protein analysis. The main contributions of this study are: 1) ability for analyzing short time stimuli response of adherent cells; 2) integration of cell culture, stimulation, with protein analysis in one device; 3) multiple time points are obtained from a single experiment.

Keywords: adherent cell, early response, In-Cell Western

1. INTRODUCTION

Some protein response events involved in cell signalling in mammalian cells exhibit fast transient responses [1]. Analysis of such responses requires very short treatment times and well-controlled and reproducible stimulus conditions. The pathways can be difficult to reproducibly probe with conventional laboratory techniques as even small fluctuations in the manual handling become significant at short times. Microfluidic systems offer the potential for reproducible and automated analysis with good control over experimental parameters such as volume, concentration, and temperature. Our overall aim is to achieve cell culture, cell stimulation, and protein analysis in microfluidic devices and thereby expand greatly the number and reliability of experiments designed to collect information on signalling pathways.

2. EXPERIMENTAL

The microfluidic device consists of a fluidic channels defined in a poly(dimethylsiloxane) (PDMS) layer fabricated by soft lithography and subsequently bonded to a glass slide. Schematics of the device and the fluidic system are shown in Fig.1. All fluidic features are 300 μm deep and 50 μm wide. A typical process flow in these experiments starts with cell culture. Prior to seeding, the device was sterilized and filled with culture media. Suspended cells were seeded at ~ 10^6 cells/mL. The cells were cultured at 37°C in a humidified incubator with 5% CO_2 in air. After 5-6 days cell culture in the devices, fresh cell medium and stimuli to cells (cytokines or growth factors) under controlled conditions of concentration, time, and temperature were added. An air segmentation of liquid streams was used to separate the fresh medium and stimuli, and to trace the position and time of the head of the stimuli treatment. The time needed for the head of the stimuli to pass a specific positions in the device and reach the end of the channel defined the stimulation time for cells in that position. Consequently, spatial

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positions along the channel corresponded to time points. After the stimulus, the cells were fixed and a primary antibody added. After incubating for several hours, a fluorescently labelled second antibody was added with an additional one hour of incubation. Finally, the fluorescence ass measured along the length of the channel.

3. RESULTS

Three types of cell lines (Hela cells, HT-29 cells, and HepG2 cells) have be tested for cultivating in the devices. Fig.2 shows the three cell types growing in the devices after 2 days. Hela cells and HT-29 cells are cultured in batch reactor mode. HepG2 cells are grown under feed-batch model.

One advantage of microfluidic devices used for the immunocytochemical assay over the convention methods is reducing the incubation time for the antibody binding [2]. Fig.3 shows the p-AKT signal of the HT-29 cells under insulin-like growth factor (IGF) treatment by different incubation times. Under flow condition, the cell signals measured by the fluorescence after half an hour or one hour incubation of the primary antibody are comparable with that after overnight incubation without flow, but the cell signals are relatively lower after half an hour or one hour incubation of the primary antibody without flow. The main reason is the enhanced mass transfer in the microfluidic device relative to conventional stationary batch procedures.

Fig.4 shows In-Cell Western results of AKT, ERK, and JNK signalling in HT-29 cells stimulated with IGF, epidermal growth factor (EGF) and tumour necrosis factor-alpha (TNFα) for different times in the microfluidic devices and 96-wells. Stimulation of cells performed in the device results in pathway activations comparable to those observed when using conventional methods. However, the error bars of the microfluidic data are relatively smaller than in the conventional method, which suggests that the microfluidic system provides better time control and correspondingly reproducible analysis of fast transient responses in the cell signalling pathways. Furthermore, additional information about the early stage of the cell signal responses are achieved by this technique.

4. CONCLUSIONS

HT-29 cells have been studies for cytokine early response under short time stimuli treatment. The AKT, ERK, and JNK signalling stimulated with IGF, EGF, and TNFα in the micrfluidic device are comparable with those from conventional methods. However, a single microfluidic experiment produces a complete temporal response curve, while conventional approaches would have required multiple experiments and manual immunocytochemical assays by standard 96 well and pipetting techniques.

ACKNOWLEDGEMENTS

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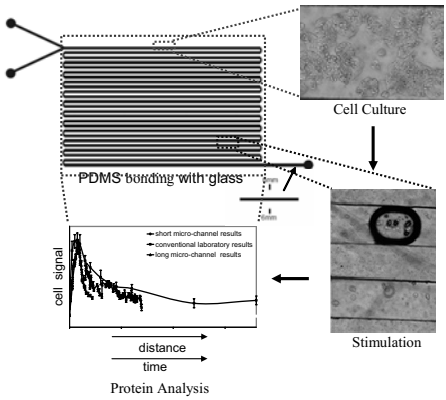

Figure 1. Schematic of the fluidic system layout

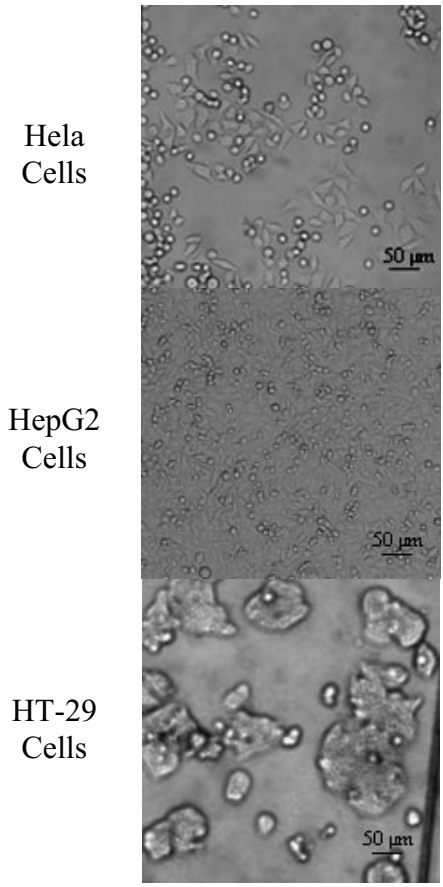

Figure 2. Cell growing in the device after 2 days

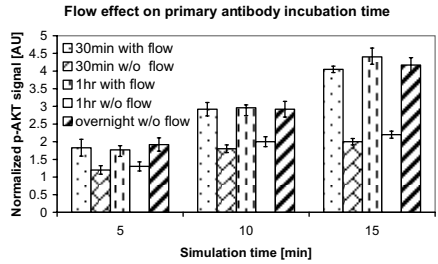

Figure 3. p-AKT signal of HT-29 cells under IGF treatment by different incubation times

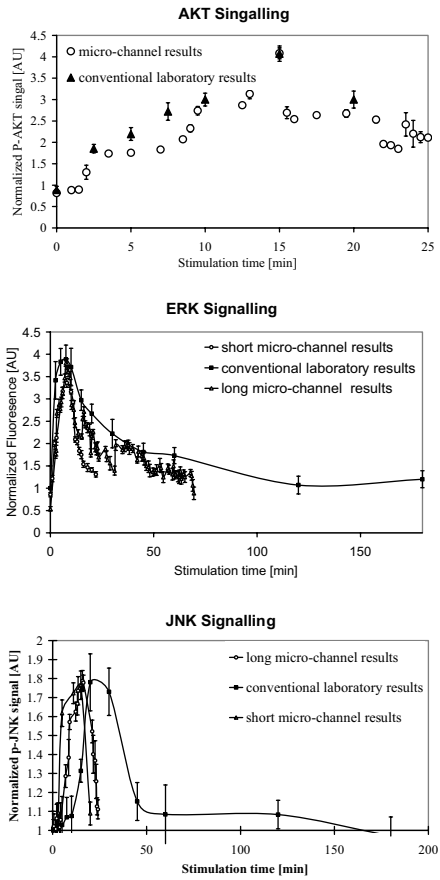

Figure 4. p-AKT, p-ERK and p-JNK signalling in HT-29 cells under IGF, EGF and TNFα treatment for different times