A FULLY INTEGRATED CELL-BASED CYTOTOXICITY, GENE AND PROTEIN EXPRESSION ANALYSIS PLATFROM

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ABSTRACT

We demonstrate a novel, user-friendly biological microprocessor for highly efficient cell capture, culture, treatment and analysis. The device provides a multipurpose platform for a wide range of molecular and cell biology methods. As pilot application, we implement a cell-based cytotoxicity assay as well as gene and protein expression studies in both adherent and non-adherent mammalian cells. The gene expression studies are performed on mRNA level based on real-time Nucleic-Acid-Sequence Based Amplification (NASBA) while the protein expression analysis uses fluorescent immunostaining.

KEYWORDS: Cell-based assays, Cytotoxicity, NASBA, Immunostaning, Gravity

INTRODUCTION

Microfluidic cell culture shows an enormous potential in biomedical research applications, since it can provide physiologically relevant microenvironments. However, due to the operational complexity and non-flexible functionality of current devices, the transfer of microfluidic cell-based assays into a biology laboratory is still in infant stages [1, 2].

We designed a novel, user-friendly multipurpose, microfluidic device for cell-based applications, based on gravity driven flow and cell sedimentation, as well as purely diffusive exchange of liquid reagents. The device provides a simple and versatile platform for parallelized cell capture, treatment and analysis applicable to many biological assays.

EXPERIMENTAL

The integrated microfluidic device was fabricated using standard soft-lithography. The device has the unique ability to dynamically configure and execute a sequence of basic unit operations required for a single assay in each of its 64 processing modules (Fig. 1). Such a module can perform and replicate an individual assay in 8 separate reactions. Each reaction is performed within a processing chamber exhibiting a trench structure. The reagents and samples are loaded with standard-pipettes. The key characteristic of the trench structure is the ability to efficiently capture cells through sedimentation and the mere diffusive loading, mixing and replacement of liquids through a controlled flow over the top of the trench (Fig. 2).

978-0-9798064-2-1/μTAS2009/$20

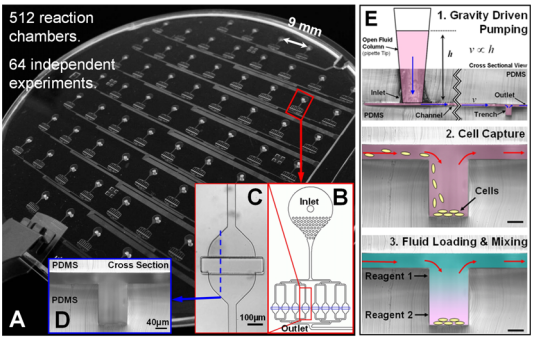

Figure 1. The array (A) consists of 64 modules (B). Each of them contains 8 "lab-in-a-trench" units (C,D) running in parallel. Flow is generated and controlled simply by the pressure head generated by the open fluid column (E).

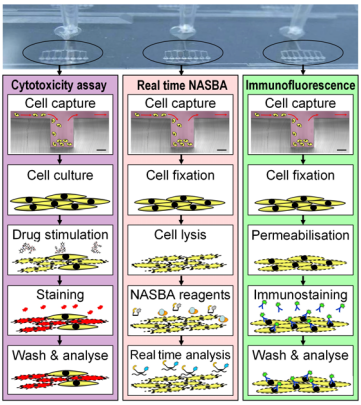

Figure 2. Schematic view of cell-based applications performed on a single integrated microfluidic device. Paclitaxel cytotoxicity assay, NASBA based gene expression analysis and immunofluorescence based protein expression analysis were performed on adherent and non-adherent mammalian cells.

RESULTS AND DISCUSSION

1-Cytotoxicity assay Paclitaxel is a well-known mitotic inhibitor used as anticancer agent that reduces the growth and proliferation of cancer cells. 64 processing chambers were loaded with approximately 50 HeLa cells each. The cells were then stimulated with eight different concentrations of Paclitaxel. Cell viability was quantified using Propidium iodide (P.I) and Calcein AM (C.AM) fluorescent staining. As expected, the number of dead cells increased with the dose of Paclitaxel (Fig. 3).

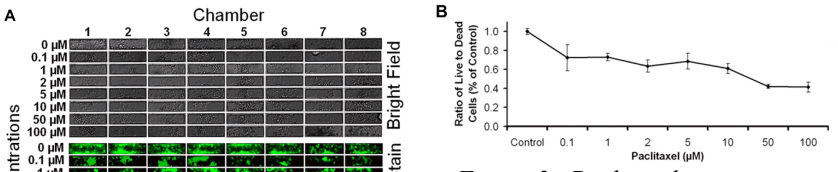

Figure 3. Paclitaxel cytotoxicity assay. HeLa cells were trapped and cultured on-chip and exposed to different concentrations of the chemotherapeutic drug Paclitaxel (A). The cytotoxic effect of Paclitaxel on HeLa cells is dose dependent (B).

2-Real time NASBA Since estrogen receptor alpha (ESR1) is a major oncogene in breast cancer, we used ESR1 positive MCF7 breast cancer cells to demonstrate multiplexed, NASBA-based gene expression analysis with primers and molecular beacons specific to ESR1 and to the house keeping gene PPIB (Fig. 4). The device is capable of performing an integrated real time NASBA protocol from as few as five cells only.

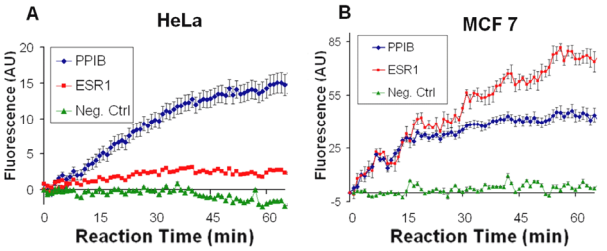

Figure 4. Real-time NASBA. Multiplexed NASBA for ESR1 and the housekeeping gene PPIB (A-B). The reaction shows a clear difference between ESR1 positive MCF7 and ESR1 negative HeLa cells.

3-Immunofluorescence ESR1 protein is known to be localized in the nucleus of MCF7 cells. MCF7 and ESR1 negative HeLa, 59M and non-adherent MM cells were captured, fixed, permeabilised and immunostained on the chip (Fig. 5). The experiments with anti-ESR1 antibody display a specific nuclear staining in MCF7 cells.

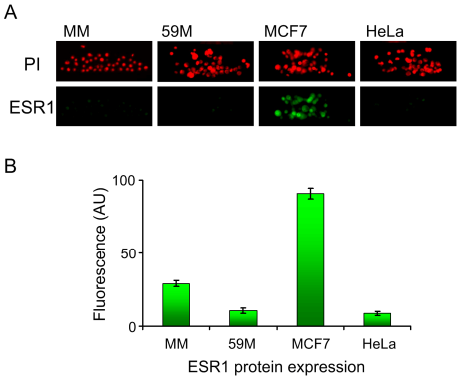

Figure 5. Estrogen receptor alpha (ESR1) immunostaining. MM, 59M, MCF7 and HeLa cells on-chip, which are fixed, permeabilised and stained (red) using Propidium iodide (PI) and anti-ESR1 mouse antibody (ESR1) followed by a secondary Alexa488 (green) labeled anti-mouse antibody. MCF7 cells show specific nuclear staining for ESR1(A). ESR1 staining quantification (B).

CONCLUSIONS

We developed a microfluidic device capable of a wide range of molecular and cell biology assays. To demonstrate its versatility we performed a cell cytotoxicity assay as well as gene and protein expression studies. This powerful device can be used from routine applications in a biology laboratory to high content screenings.

ACKNOWLEDGEMENTS

This work was supported by the Science Foundation Ireland, 05/CE3/B754.

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