MICROFLUIDIC PLATFORM FOR INTEGRATED ANALYSIS OF CYTOTOXICITY, GENE AND PROTEIN EXPRESSION IN CELLS

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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].

Here we demonstrate a novel, user-friendly biological microprocessor with highly efficient cell capture, culture, treatment and analysis, which has a multi-purpose platform for a wide range of molecular and cell biology methods. As pilot applications, we implement a cell-based cytotoxicity assay as well as gene and protein expression studies simultaneously in both adherent and non-adherent mammalian cells on a chip. The gene expression analysis utilizes the RNA Nucleic-Acid-Sequence Based Amplification (NASBA) while the protein expression uses fluorescent immunostaining.

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.

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 housekeeping gene PPIB (Fig. 2. A,B). The device is capable of performing an integrated real time NASBA protocol from as few as five cells only.

ESR1 protein is also 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. 2. C,D). The experiments with anti-ESR1 antibody display a specific nuclear staining in MCF7 cells.

In summary, we designed a novel, user-friendly multipurpose, cell-based device and demonstrated its capability to perform cytotoxicity assays, real-time gene expression analysis and protein expression assays. The device can be used from routine applications in a biology laboratory to high content screenings.

REFERENCES