ARRAYED CAPTURE, ASSAYING AND BINARY COUNTING OF CELLS IN A STOPPED-FLOW SEDIMENTATION MODE

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ABSTRACT

In this work we present a centrifugal microfluidic platform for highly efficient trapping, staining and identification of biological cells. V-shaped geometric traps are used to captured mixed populations of cells under stagnant flow conditions. We demonstrate the capability to discriminate cells based on surface markers (EpCAM) as well as on intracellular markers (ESR1). Captured cells are arrayed in single cell ordered pattern and align in a well-defined focal plane which greatly facilitates the readout of cell-based assays.

KEYWORDS: Centrifugal Microfluidic, Single Cell Capturing

INTRODUCTION

Microfluidic systems offer a unique approach to handle biological cells and to perform cell-based assays for biological studies as well as for clinical diagnostics. Devices for single cell capture are of special interest since they allow to study individual cells and their direct responses to various conditions. Single cell capture also allows to discriminate and count individual cells in subpopulations of mixed cells. Several devices for cell capture have been developed in the past, using, for example, capture elements in pressure driven systems to enable cell fusion [1] or centrifugal systems with radially sloped channels and pockets in the side walls [2]. Centrifugal microfluidic systems are especially suited for cell capturing applications since they can take advantage of the density difference between cells and surrounding medium to sediment cells. Furthermore, a wide range of liquid handling functions such as metering, valving and mixing have already been shown in the past [3]. Our system utilizes an array of V-cup elements to capture cells under stagnant flow conditions on a centrifugal platform, thus overcoming the typically low capture efficiency of pressure driven systems.

THEORY

The system presented here utilizes our previously introduced V-cup array structure for single cells capture on a centrifugal microfluidic disc [4]. In brief, cells sediment in a chamber on the disc under stagnant flow conditions due to the centrifugal force into an array of V-shaped capturing elements. Since the sedimentation takes place with the liquid bulk at rest, i.e., in the absence of flow (lines), the capture efficiency of this system is much higher than that of typical flow driven systems. Pressure driven systems are typically characterized by a rather low capture efficiency due to the continuity of streamlines which diverge around obstacles and hence drag cells around the capturing elements. This can be reduced to a certain extent by introducing gaps in the capturing elements or between capturing elements and the bottom of the chip. However, this approach adds significant complexity to the micromanufacturing process.

A sharply peaked single cell occupancy distribution can be achieved by scale-matching of the size of the capture elements to the size of the cells [1]. Once cells are captured in the V-cup array, the reagents in the capturing chamber can be easily altered exposing the cells to a variety of conditions (e.g., antibodies, stains or washing buffers). The working principle is shown in Fig. 1.
EXPERIMENTAL

All discs used in this work have been made in PDMS (Dow Corning, USA). To this end molds have been fabricated using standard lithography. Briefly, a 30-µm layer of SU8-3025 (Microchem, USA) has been spin coated on silicon wafers and structured with the V-cup array. Subsequently, two layers of 100-µm dry film resist (WBR 2100, DuPont, USA) have been laminated and structured to create the reservoirs. Prior to casting the PDMS, a hydrophobic coating has been applied to the molds to facilitate release of the PDMS. Subsequently, PDMS was mixed in a ratio of 1:5 (curing agent to base), poured onto the mold and cured at 70 ºC. The substrates were sealed by bonding to a PMMA disc coated with partially cured PDMS in a mixing ratio of 1:20 and left in the oven at 70 ºC over night. Prior to use, the PDMS discs were stored in vacuum for at least 20 min to ensure bubble free filling of the array.

Three different cell lines were used in this work: HeLa, MCF7 and RPMI-8226. The cells were harvested from cultures immediately prior to the experiments. Two different assays have been used in this work. The first is an assay to discriminate cancer cells (MCF7) from a background of plasma cells (RPMI-8226) by detecting the surface protein EpCAM. The second assay uses an intracellular marker (ESR1 - Estrogen Receptor α) to differentiate cells. The following staining protocol was used: Once captured, cell were fixed using a solution of 4 % formaldehyde (Sigma-Aldrich, Ireland) and cell were permeabilized using a solution of 0.05 % Triton X100 (Sigma-Aldrich, Ireland) in PBS Buffer. Nuclear DNA was then stained using Propidium Iodide (Invitrogen, USA). For the EpCAM assay, the cells were subsequently exposed to anti-EpCAM antibody from goat (BAF960, R&D Systems, USA) followed by an incubation with a fluorescently labeled anti-goat antibody (F0109, R&D Systems, USA). In the case of the ESR1 assay, cells were first incubated with anti-ESR1 antibody from mouse (SC-8002, Santa Cruz Biotech, USA) and then incubated with fluorescently labeled anti-mouse antibody from goat (A11001, Invitrogen, USA). Including the washing and blocking steps, the liquid in the capturing chamber is exchanged 18 times during this assay. Images have been taken using a fluorescence microscope (IX 81, Olympus, Japan).

RESULTS AND DISCUSSION

Identification of cancer cells among plasma cells: A population of RPMI-8226 plasma cells has been spiked with a small amount of MCF7 breast cancer cells. 2 µl of the cell suspension were then introduced into the disc and cells were captured in the V-cup array. Next, the cells were stained using the EpCAM staining protocol described above. Figure 2 shows the results of these experiments. MCF7 cells can easily be distinguished from the population of plasma cells.
Figure 2: RPMI plasma cells spiked with a small quantity of MCF7 cancer cells have been captured in the array (a). The nucleus of all cells has been stained with PI (b). MCF7 cells are identified using anti-EpCAM IgG and a secondary FITC-labeled detection antibody (for better discrimination between anti-EpCAM and anti-ERa, the fluorescent signal from anti-EpCAM IgG has been false colored in blue)(c). All scale bars: 100 μm.

On-chip Estrogen Receptor α expression analysis: In order to demonstrate the capability to classify cells based on intracellular markers, a population of MCF7 cells has been spiked with HeLa cells. The mixed sample was loaded onto the disc and cells were captured in the V-cup array. Staining protocols were performed as described above. Figure 3 shows the captured cells after staining. MCF7 and HeLa cells can easily be discriminated.

Figure 3: A mixed population of HeLa and MCF7 cells has been trapped in the array. Part (a) shows a bright field image of the trapped cells, (b) a fluorescent image of all cells with PI stained nuclei (red), and (c) the ESR1-expressing MCF7 cells which are selectively stained (green) by a FITC-labeled anti-ESR1 antibody. By comparing images (b) and (c), the ESR1-negative (HeLa) cells can easily be identified (white circles). Scale bars: 100 μm.

CONCLUSION
In summary, we demonstrate the capability of our centrifugal V-cup array platform to capture mixed populations of biological cells and discriminate these based on either membrane proteins such as EpCAM or intracellular proteins such as ESR1. The arrayed display of single cells in one focal plane greatly facilitates detection and readout. Future work will concentrate on using this platform to answer clinically relevant questions such as cell identification and counting.

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