EXTRACTION OF CIRCULATING TUMOR CELLS FROM BLOOD USING ACOUSTOPHORESIS

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ABSTRACT

We present, for the first time, separation of three different prostate cancer cell lines from leukocyte fractions by means of continuous flow acoustophoresis. This flow-through separation approach, which utilize acoustophoretic forces to extract CTCs from blood has a significant advantage over affinity based methods in the sense that it comprises a non-contact and label free separation of CTCs from blood.

KEYWORDS: Circulating Tumor Cells, Acoustophoresis, Ultrasound, Cell Separation

INTRODUCTION

Extraction and analysis of circulating tumor cells (CTCs) from peripheral blood has recently emerged to monitor metastatic cancer stages and to assess therapeutic efficacy of different treatment modalities, either by cell count or by gene expression profiling [1-3]. Several strategies have been proposed such as physical [4], and affinity based approaches involving microbeads [5] or micro-posts [6]. The large variations not only in morphology but also in antigen expression among CTCs, call for novel approaches to enhance CTC enrichment. We propose a flow-through separation approach, which utilize acoustophoretic forces to extract CTCs from blood.

EXPERIMENTAL

The acoustophoresis separation device is a silicon/glass microchannel [7] having a trifurcation inlet and outlet, Fig. 1. A piezoceramic crystal actuates the separation channel at λ/2 resonance, creating a force on suspended cells directed towards the vertical center plane of the channel. Cell samples are infused via the side inlets and are laminated along the separation channel walls, while buffer media is infused via the centre inlet. At the end of the separation channel the flow is split in a 1:1 ratio between the side outlets and centre outlet. The amount of cells obtained in the central outlet fraction is determined by; the retention time in the acoustic field (i.e. flow rate), the acoustic field strength, the size of the cells, and the acoustic properties of the cells relative to the suspending medium. Thus, for a mixture of different cell types we can expect large cells (CTCs) to gather in the central outlet while smaller cells (blood cells) remain in the flow path that is directed to the side outlet fraction.

Samples of prostate cancer cell line DU145 (2.5 x 10^5 mL^-1) spiked in red blood cell (RBC) lysed whole blood (diluted 1:10), were fixated and processed in the acoustophoresis chip at different flow rates in order to map the separation efficiency. Due to large variations in morphology among CTCs occurring in clinical samples, separation efficiency for three different prostate cancer cell lines were investigated. Prostate cancer cell lines DU145, LNCaP and PC3 (2.5 x 10^5 mL^-1) where spiked in RBC lysed whole blood and processed in the chip.

To investigate any potential cell damage caused by the ultrasound, live cells from all three cell lines were run through the separation channel at low flow rate and high ultrasound intensity. After one passage through the device the percentage of viable cells was estimated by Tryptan blue staining followed by counting of viable respectively dead cells in a Bürker chamber.

Figure 1: (a) Schematic of acoustophoresis cell separation principle. Cancer cells are collected in the central outlet fraction due to a higher lateral velocity in the acoustic potential. (b) Schematic of the fluidic setup. Separation parameters are investigated by analysis of collected fractions from the outlet sample loops.
RESULTS AND DISCUSSION

Output fractions analyzed by flow cytometry reveal that a high degree of separation can be achieved between leukocytes and DU145 cells, Fig. 2. The separation efficiency of the system spans from complete removal of leukocytes for a total flow rate of 800 μL/min to full recovery of DU145 cells for a total flow rate of 320 μL/min, Fig. 3(a). The sample throughput corresponding to the above flow rates is 100 μL min⁻¹ and 40 μL min⁻¹, respectively. By elevation of the ultrasound intensity it is possible to increase the sample throughput of the device even further while maintaining the separation efficiency (data not shown).

Analysis of processed samples from three different cell lines all displayed good separation efficacy from leukocytes, Fig 3 (b). This result indicates the methods robustness regarding the expected diversity of CTCs in the peripheral blood of metastatic prostate cancer patients.

The strain inflicted on cells when passing through the acoustic field in the microfluidic chip do not seem to influence the cell viability, Fig 4. We thus support several studies that have shown that ultrasonic cell manipulation in acoustic standing wave fields is not inducing any monitorable damage to the cells.

Figure 2: Comparison of the flow cytometry data from the (a) central outlet fraction and (b) the side outlet fraction. Leukocytes are marked in pink and DU145 cells are marked in green.

Figure 3: (a) Percentage of cells in the central outlet relative to the total number of cells in the two outlets. Data ranges from complete removal of leukocytes to complete recovery of cancer cells. (b) Comparison of the collection efficiency for extraction of three different prostate cancer cell lines compared to leukocyte carry-over. (N=5)
CONCLUSION

We believe that this work contributes principal proof of concept showing that acoustophoresis has potential for use in clinical diagnostics of CTCs in cancer patients. Moreover, the modular selection criteria allows for either high clearance of blood cells or a high recovery of CTCs, depending on desired application.

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