DEFORMABILITY BASED SEPARATION OF CIRCULATING TUMOR CELLS FROM PATIENTS WITH CASTRATE RESISTANT PROSTATE CANCER

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ABSTRACT

We present a deformability based cell separation device created using the microfluidic ratchet mechanism to separate live circulating tumor cells from whole blood. This device is capable of log-4 depletion of leukocytes when tested using cultured cancer cells doped into whole blood. To evaluate this device, we separated and enumerated CTCs from 33 patients with castrate resistant prostate cancer and 6 healthy controls, and compared the results in simultaneous experiments performed using the CellSearch system, the current commercial standard. The CTC capture rate is significantly higher for our device, which detected \(>5\) CTCs in 75.5% of patients with an average count of 235, while the CellSearch system detected \(>5\) CTCs in 36.3% of patients with an average count of 104.

KEYWORDS: Circulating tumor cells, Label-free, Deformability, Cell sorting

INTRODUCTION

CTCs have been implicated as potential seeds of cancer metastasis and have strong prognostic and diagnostic value in cancer therapy. The primary challenge in CTC characterization is their extreme rarity in circulation, where they may be found at a frequency as low as one in \(10^7\) leukocytes, the primary contaminant cell type. Conventional strategies employ CTC immunoenrichment that is highly selective but may fail to enrich for CTCs with poor antigen expression. Alternatively, CTCs are likely to exhibit morphological characteristics that distinguish them from leukocytes [1], which enable separation using label-free biophysical mechanisms [2]. Recent label-free separation methods, including hydrodynamic chromatography, inertial flow, and dielectrophoresis, primarily distinguish CTCs from leukocytes based on size alone, which may results in low enrichment due to the size overlap between CTCs and leukocytes [3-7]. Micropore filtration is a simple method to separate cells based on a combination of size and deformability. However, this approach is limited by clogging, where cells accumulated in the pores modifies the resistance of the filter and filtration force applied to incoming cells in an unpredictable way.

MECHANISM AND DESIGN

We developed the microfluidic ratchet mechanism enabling highly selective deformability based cell separation without clogging [8,9]. This mechanism relies on the asymmetrical force required to deform single cells through funnel-shaped constrictions. As shown in Figure 1, when cells are pushed through...
such constrictions, cells with a certain combination of size and deformability will transit through the constriction only in the forward flow but are unable to return in reverse flow. Oscillatory flow through multiple sets of these asymmetrical constrictions enables a continuous separation process based on a combination of cell size and deformability. A key advantage of this process is that the sample cells come into contact with the filtration microstructures only momentarily, and thereby greatly limiting the potential for clogging and adsorption.

The deformability based cell separation device consists of a 32x2048 matrix of funnel constrictions along with supporting microchannels for flow control. The openings of the funnel constrictions are gradually reduced from the bottom row to the top row, ranging from 18 µm to 2 µm. Cells enter at the bottom-left of the funnel matrix and are driven by a rightward flow simultaneously as a vertical oscillatory flow. Each cell traverses through the funnel matrix in a step-wise diagonal path until reaching a limiting funnel size, at which point the cell moves horizontally towards the outlet (Figure 2). CTCs are the least deformable cells and reach their limiting funnel size relatively quickly. Leukocytes are more deformable and travel to a smaller funnel region. Finally, erythrocytes are extremely deformable and exit through the top of the matrix.

METHODS
This device was fabricated in a single layer of PDMS bonded to a glass microscope slide. Prior to the experiment, the device was filled with PBS and 0.2% Pluronic to prevent nonspecific adsorption. We used whole blood doped with small concentrations of UM-UC13 human bladder cancer cells stained with Calcein AM to characterize the device. Results were analyzed visually using microscopy. We characterized the performance of the device in terms of yield (% of UM-UC13 cells collected in outlet), enrichment (initial cancer cell ratio/final cancer cell ratio), and throughput. Patient samples were processed directly within 24 hours after blood is drawn. Separated samples are then stained for cytokeratin (CK), EpCAM, CD45, and DAPI and scanned by confocal microscope with analysis of the emission spectrum.

RESULTS AND DISCUSSION
Using different oscillation flow rates, the specificity (enrichment) and sensitivity (yield) of this mechanism can be adjusted. Specifically, at 90% yield, an enrichment >5,000 can be achieved; while at the enrichment of 13760, yield can reach >76%. These values rival some of the best-reported results in this field. Cell morphology of UM-UC13 cells and leukocytes were analyzed and theoretical enrichments by using size based separation method were calculated. By comparing the enrichment of separation based on size only and separation based on size and deformability, we can see that deformability contributes to a ~10 to 100 fold increase in its ability to deplete leukocytes (Figure 4).

We used the microfluidic ratchet device to enumerate CTCs from 33 patients with castrate resistant prostate cancer and 6 healthy control samples in a head-to-head comparison with the CellSearch system. Captured cells were stained and identified with spectral analysis (Figure 5 and 6). This process showed highly distinct spectral patterns between CTCs (identified as DAPI+CK+EpCAM+/CD45-) and leuko-
cytes (DAPI+CD45+). The microfluidic ratchet device found CTCs (>5) in 25/33 patients with an average count of 235, while CellSearch found CTCs (>5) in 12/33 with an average count of 104 (Figure 7). In the 20 patients where CTCs were not found by CellSearch, 13 were found with more than 5 CTCs by the microfluidic ratchet. In fact, 5 of these patients had CTC counts from 60 to 1519. These results suggest that deformability based separation may select for distinct populations of CTCs than traditional antigenic selection based on EpCAM.

**CONCLUSION**

We present a deformability based cell separation device capable of separating live circulating tumor cells from whole blood. System characterization using healthy blood doped with cultured cancer cells demonstrates significantly greater selectivity than separation based solely on size. This device successfully isolated circulating tumor cells from cancer patients with castrate-resistant prostate cancer and demonstrated significantly better performance than the CellSearch system. Our results suggest that deformability based separation may select for distinct populations of CTCs than antigenic selection.

**REFERENCES**


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