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Two-step acoustofluidic cancer cell enrichment

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Introduction

We have previously proposed and evaluated acoustophoresis for the enrichment of circulating tumor cells (CTC) and clusters thereof from the blood of patients with prostate cancer [1]. In that protocol, the cells were fixed and preserved in paraformaldehyde before processing and were thus non-viable. To enable future enrichment of also *viable* CTCs from patients, we have in this study investigated the ability to discriminate cancer cell line cells from sub-groups of viable white blood cells (WBC). We found that acoustophoresis alone has poorer performance for viable cells than for fixed cells. Therefore, we added a second step of acoustic gradient separation wherein cells migrate into a medium of increasing acoustic impedance. The resulting separation results in >80% of cancer cells reaching the target outlet while 99.8% of all WBCs are removed.

Background

CTCs are shed into the blood from primary tumors or metastatic lesions. It is well established that high circulating tumor cell counts are associated with poor outcomes and lower survival in metastatic cancers [2]. The FDA-approved CellSearch assay, considered the gold standard to enumerate CTCs, relies on magnetic bead antibody capture. However, it cannot detect cells with low expression of the surface molecule EpCAM. Thus, there's a critical need for alternative approaches to isolate circulating tumor cells. Apart from the enumeration of CTCs to monitor disease progression, there is an interest among cancer biologists to recover viable CTCs from patients to elucidate the underlying mechanisms of metastasis. To achieve this, we have investigated the possibility of using a two-step sequence of acoustic separations.

Experiments

We collected blood from healthy donors, and red blood cells (RBC) were chemically lysed resulting in a population of WBCs. Samples were constructed by mixing cultured cancer cells with WBCs. Cells were either paraformaldehyde-fixed or non-fixed (viable). Two modules for acoustic separation were employed sequentially. **Figure 1(A)** illustrates step 1, which is based on the notion that cancer cells migrate faster than WBCs when exposed to sound [3-5], predominantly due to their larger size. In step 2, the cells from the central outlet, were resuspended in a new medium and subjected to iso-acoustic focusing, wherein cells of low effective acoustic impedance are blocked from entering the central part of the flow, **Fig. 1(B)**.

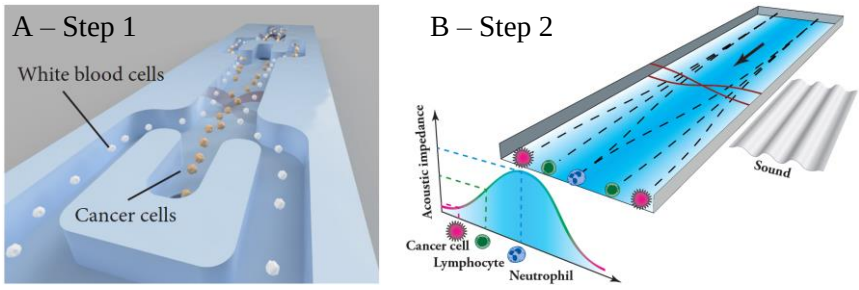

Figure 1: Separation modules. (A) In step 1 cancer cells are separated by their mobility in a saline solution by conventional acoustophoresis. (B) In step 2, contaminating WBCs of high effective acoustic impedance are removed by iso-acoustic focusing.

Results and Discussion

First, we investigated the cancer cell separation efficiency for MCF7 breast cancer cells in step 1 for fixed and viable cells. The central fraction contains ~80% of all recovered cancer cells for both cases, while the contaminating WBCs in the central fraction increase dramatically for non-fixed samples, **Fig 2(A)**. Looking at WBC sub-groups, we found that the main contamination is granulocytes, **Fig. 2(B)**. Granulocytes are in general smaller than MCF7 cells, but their density is considerably higher. Therefore, a large fraction of these cells have acoustic mobilities that overlap with the cancer cells. Flow cytometry analysis (not shown here) indicates that the fixation protocol makes the WBCs smaller and more uniform in size, while MCF7 cells are seemingly unaffected by fixation, and this can explain why separation efficiency is higher for fixed cells.

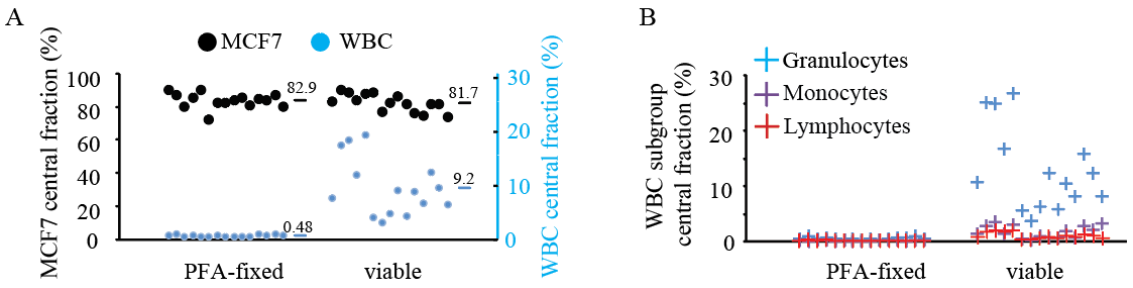

Figure 2: Step 1 acoustophoretic separation of MCF7 cancer cells from WBCs for fixed vs non-fixed (viable) cells. (A) Central fraction of cells comparing MCF7 cells and total WBCs. (B) Contaminating WBC subgroups in central fraction.

To remove the contaminating granulocytes, we ran cells through iso-acoustic focusing with the idea that granulocytes could penetrate further into an acoustic impedance gradient compared to cancer cells [6]. **Figure 3(A)** shows the fraction of MCF7 cancer cells that reach the target side outlet and the corresponding fraction of granulocytes that reach the central outlet when the acoustic impedance of the central flow stream is increased by increasing the concentration of the solute molecule iodixanol. For 16% iodixanol, the barrier blocks nearly all MCF7 cells while ~97% of the granulocytes penetrate the barrier and reach the central outlet.

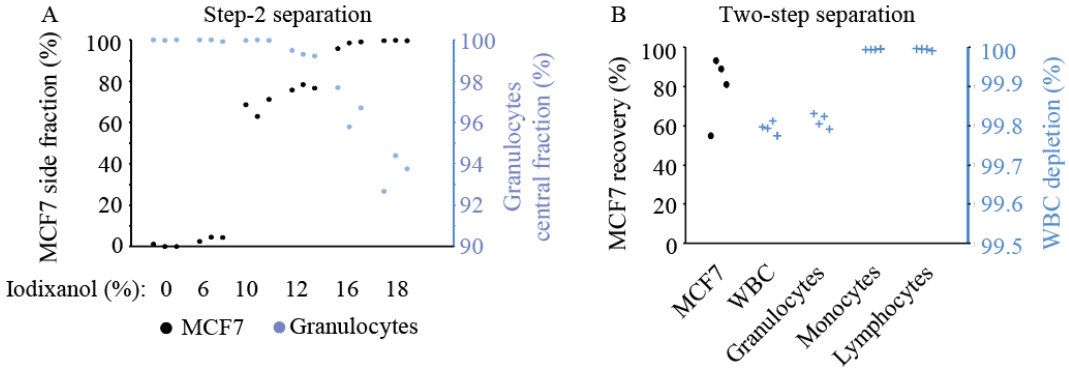

Figure 3: (A) The fraction of MCF7 cells that are blocked by the acoustic impedance barrier and end up in the side outlet and the corresponding fraction of granulocytes that penetrate the barrier and end up in the central outlet, for increasing iodixanol concentration in the central medium. (B) The final recovery (output vs input) of MCF7 cells after the two-step separation and the corresponding depletion of WBCs, by subgroup.

We then constructed samples of 500 µL containing 5×10^5 WBCs and $2\cdot10^4$ MCF7 cells and ran them through the two-step separation procedure. When comparing the output to the input, we measured a mean recovery of ~80% and a WBC depletion of 99.8% which is on par with the one-step procedure for fixed cells, **Fig. 3(B)**.

Conclusions

With this work, we demonstrate that multistep acoustic separation of cells presents opportunities to isolate rare cells with high recovery and selectivity based on a panel of biomechanical properties.

Acknowledgments

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