A Microfluidic Platform for Single-Step Isolation and Mechanotyping of Ovarian Cancer Cells from Patient Samples

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1. Purpose
The mechanical properties of cells are affected by a wide variety of factors, including cell type, cell viability, and numerous disease states. For example, in metastatic ovarian cancer, variation in cell stiffness is associated with differences in the aggressiveness and invasiveness of the disease [1]. As our group has previously demonstrated, the mechanical properties of cells also serve as biomarker suitable for high throughput, label-free cell sorting applications [2–5]. Using our microfluidic platform illustrated in Figure 1, we will isolate rare cancer cells from patient samples based on their mechanical properties, a label-free approach which allows for the analysis of a patient's metastatic cells via both functional and gene expression assays. By further taking advantage of the differences in stiffness between various cancer cell subpopulations we show the feasibility of a device for single step cancer cell isolation and mechanotyping. In addition to the diagnostic information provided by cancer cell isolation, such a device will also give a measure of the mechanical properties of cancer cells and information on how these properties vary among different subpopulations of metastatic cells. Given that the heterogeneity of cancer cell subpopulations is implicated in drug resistance and cancer cell stiffness is associated with disease aggressiveness we believe that this device constitutes a rapid test that could provide important prognostic as well as diagnostic information while simultaneously preparing high-purity samples of rare metastatic cells suitable for downstream analysis.

2. Methods
Before sorting, cells were placed in suspension using standard cell culture protocols. All sorting experiments were performed on an inverted microscope with inlet flows provided by syringe pumps. After sorting, cells were collected and the degree of sorting was quantified by one of two methods. For cell line sorting experiments the different cell lines were dyed before the experiment using cell tracking probes of different colors, allowing the makeup of the sorted populations to be quantified using flow cytometry. Primary cells will be attached to well plates, fixed and stained using anti-transglutaminase-2 (TG2) antibodies and standard immunocytochemistry techniques. The fluorescence intensity of the individual cells in each sorted population was then measured using microscopy, allowing for estimation of the degree of TG2 expression by the individual cells. Given that TG2 is a marker for malignancy in ovarian cancer these measurements allowed us to quantify how many malignant cells were successfully captured in each sample [6].

![Figure 1](Image 74x203 to 254x319)

**Figure 1. Device overview.** Our device consists of a microchannel containing small, diagonal constrictions. The size of these constrictions is chosen so that cells must deform to pass through them. During this deformation the cells are deflected perpendicular to the flow in a manner dependent on their mechanical properties.

![Figure 2](Image 47x131 to 268x202)

**Figure 2. Enrichment of HEY and OVCAR-3 cells.** We define enrichment factor to be the ratio of target to other cells in a population divided by the same ratio before sorting (higher enrichment factor implies better sorting). In our initial experiments we have demonstrated an enrichment factor >15 for OVCAR-3 cells in our stiffest sample and an enrichment of ~2 for HEY cells in our second to softest outlet. Very few cells were collected from the softest outlet, so the enrichment factors for that sample were not calculated.

3. Results
In our initial studies, we have used our microfluidic platform to separate cell lines representative of ovarian cancer cells with varying degrees of metastatic potential. HEY cells have a mesenchymal, highly invasive phenotype similar to metastatic cells in ovarian...
cancer whereas OVCAR-3 cells have an epithelial phenotype more representative of cells in a solid tumor. As shown in Figure 2, our device has succeeded in doubling the ratio of HEY cells to OVCAR-3 cells present in the second to softest sorted sample.

4. Conclusion

In this work we have proposed a device for single-step isolation and mechanotyping of ovarian cancer cells from liquid patient samples. In our initial experiments we have demonstrated our ability to selectively enrich ovarian cancer cells based on their metastatic potential. To demonstrate the utility of our platform for both diagnostic and prognostic purposes we will also demonstrate enrichment in patient samples. By enabling cheap, label-free isolation of cancer cells with simultaneous detection of cancer phenotype we believe that our microfluidic platform could be a valuable clinical tool which could provide clinicians with valuable data while lowering the cost of downstream tests.

References


