DEFORMABILITY CYTOMETRY: APPLICATIONS IN CLINICAL CANCER DIAGNOSTICS

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ABSTRACT

A mechanical biomarker would be an attractive label-free classifier of cells for clinical diagnostic applications. However, the adoption of this biomarker has been slow due to the limited statistical confidence and power provided by low throughput techniques. Here, we demonstrate a high-throughput (2,000 cells/second) tool employing a unique combination of inertial focusing, hydrodynamic forces, and automated image analysis. Further, we demonstrate the potential of the mechanical biomarker in a clinical cancer diagnostic role in analyzing pleural effusions and in a correlative study investigating the aggressiveness of patient-derived melanoma cell lines in relation to deformability.

KEYWORDS: Inertial Microfluidics, Deformability Cytometry, High-Throughput, Single-Cell, Cell Mechanics, Biomarkers

INTRODUCTION

Recent work has shown that mechanical biomarkers relating to cytoskeletal and nuclear structures can be useful label-free identifiers of cell states and properties such as metastatic potential, cell cycle, and leukocyte activation. Clinically, a label-free measure of metastatic potential could assist in diagnosis and treatment decisions. Previously, we have demonstrated the deformability cytometer: a platform technology capable of high-throughput (> 2000 cells/second) single-cell mechanical measurements. The high-throughput capability allows application of a mechanical biomarker to complex clinical samples, beyond basic biophysics studies, due to the higher statistical confidence accompanying a larger sample size approaching that of conventional flow cytometry [1]. Here, we have applied this tool to cancer diagnostics in a large set of clinical isolates. We envision a label-free and low cost screening assay to complement conventional manual cytology analysis methods.

THEORY

The microfluidic device (Figure 1A) utilizes straight and curving asymmetric channels to position cells at a single mid-plane prior to entering the interrogation region. At the junction, converging streamlines create a purely extensional flow pattern in which cells are exposed to a continuous deformation force. The observed strain is a measure of the intrinsic cytoskeletal, nuclear, epigenetic, and other bio-structural components of the cell.

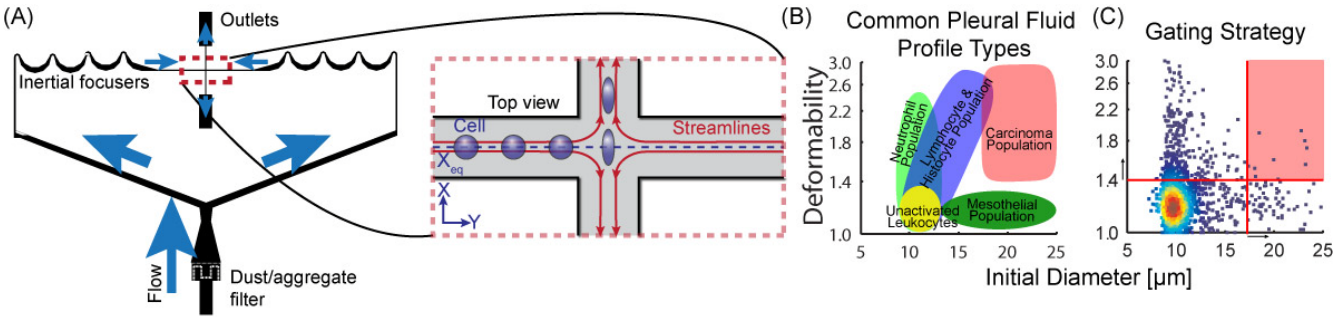

Figure 1: (A) Device schematic with inset of the continuous deformation region. (B) Common clinical profiles from pleural fluid samples. (C) Gating strategy used for testing patient sample profiles for malignancy.

EXPERIMENTAL

The microfluidic device was molded from polydimethylsiloxane (PDMS) replicated from silicon wafers made using standard photolithographic methods. At the junction region the channel aspect ratio is approximately 1:2 with channel dimensions of 30μm height x 67μm width. Pleural fluids are processed with red blood cell lysis buffer prior to assay to remove red blood cells. Cell suspensions of approximately 200,000 cells/mL are injected through polyetheretherketone

(PEEK) tubing through channels by syringe pump. Image acquisition is performed with a Phantom v7.3 high speed camera mounted on an inverted microscope. Deformation events at the junction region are recorded using a 10x objective. Finally, image analysis is performed using a custom MATLAB script on the UCLA ATS Hoffman2 cluster.

RESULTS AND DISCUSSION

To date, we have assayed 47 patient pleural effusion samples, averaging over 2,000 single-cell measurements per sample. Cell extracts from pleural effusions are routinely used for metastatic cancer screening in clinical practice. Disseminated cancer cells that collect in this fluid are predominantly from primary tumor sites such as the blood, breast, lung, gastrointestinal track, and ovaries [2]. Deformability cytometry profiles are compared to cytopathological diagnoses for these patients. Characteristic deformability cytometry profiles for cancer-negative pleural fluids correspond to a single small and stiff leukocytes population (n=22) (Figure 2) which differs dramatically from cancer-positive diagnoses containing a larger and more deformable sub-population (n=11) (Figure 3). We also are able to observe distinct inflammation profiles within the negative diagnoses: acute inflammation (n=7) (Figure 4) and chronic inflammation outcomes (n=12) (Figure 5). Interestingly, both of these types of samples were observed to possess a more deformable population of leukocytes (Figure 1B). To identify cancer-positive samples with our approach, we quantified the proportion of the cells in the high deformability (>1.4) and large initial diameter (>17μm) quadrant (Figure 1C). The unique profiles (as a function of deformability and size) for chronic inflammation cases allowed us to algorithmically identify them and lead to enhanced malignancy detection. Using this gating and analysis strategy the average profile for a cancer-positive patient yielded an expected proportion with 15.6% of cells in the region-of-interest which is a 17.9x increase over the average value for a cancer-negative patient (0.8%). From this preliminary study, we have achieved 91% sensitivity, and 86% specificity for recognition of malignancy, this level of accuracy is comparable to conventional cytology analysis methods.

We have also begun to examine a series of patient-derived melanoma cancer cell lines in which deformability values are compared with expression of the surface marker, CD271 - an indicator of the cancer-stem-cell state correlating with aggressiveness [3]. In our preliminary study using fluorescence imaging quantification, we have identified a strong correlation between the median population deformation and the CD271 level (R=0.82).

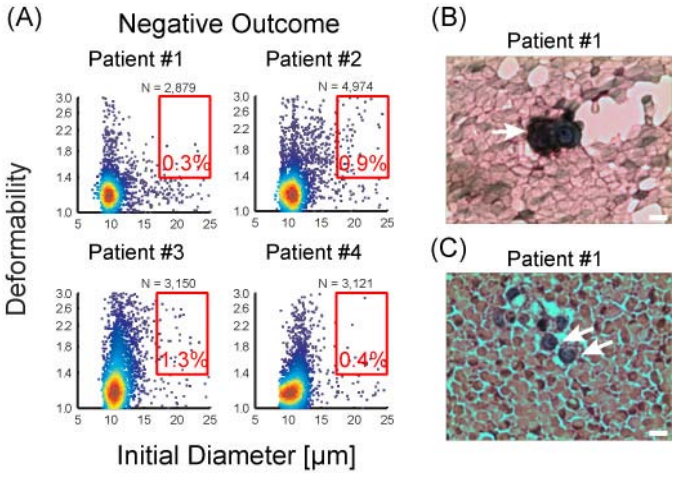

Figure 2 Negative outcome diagnosis. (A) Representative deformability profiles for patients with a negative diagnosis. (B) Cell smear (C) Cell block with arrows highlighting benign mesothelial cells. Scale bar 10 μm.

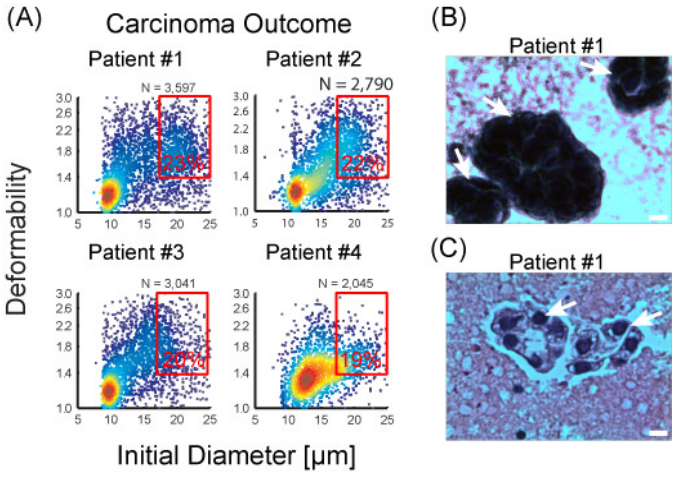

Figure 3 Carcinoma outcome diagnosis. (A) Representative deformability profiles for samples from patients diagnosed with carcinoma. (B) Cell smear (C) Cell block with arrows highlighting cancer spheroid formations. Scale bar 10 μm.

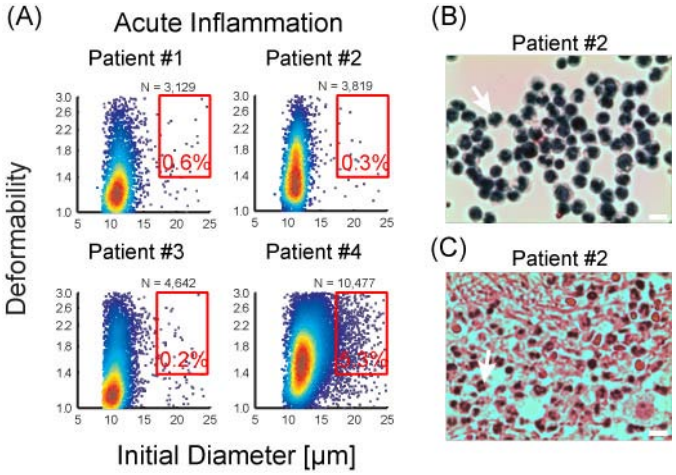

Figure 4 Negative outcome diagnosis with observed acute inflammatory response. (A) Representative patient deformability profiles for acute inflammation. (B) Cell smear (C) Cell block with arrows highlighting neutrophils. Scale bar 10 μm.

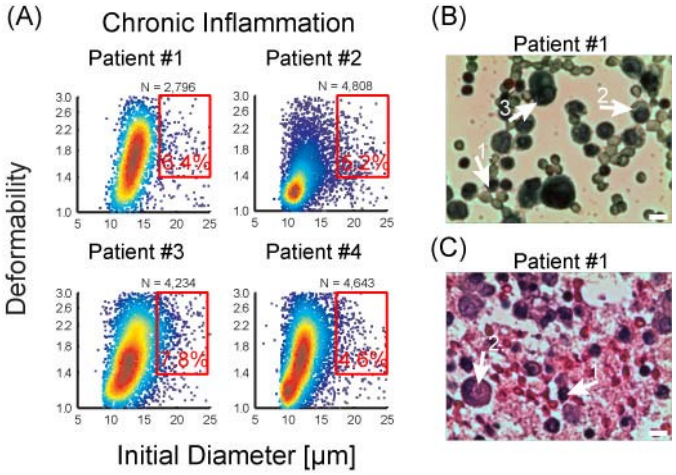

Figure 5 Negative outcome diagnosis with observed chronic inflammatory response. (A) Representative patient deformability profiles for chronic inflammation. (B) Cell smear (C) Cell block with arrows highlighting 1) lymphocytes 2) histiocytes 3) mesothelial cells. Scale bar 10 μm.

CONCLUSION

Deformability cytometry is a cost-effective label-free analysis tool that will enable the general scientific community access to the mechanical biomarker accompanied by high statistical confidence. In the clinical setting we foresee deformability cytometry as a powerful tool for cytopathology by assisting clinical decision making events for screening, staging, and treatment efficacy monitoring.

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