MICROFLUIDIC SAMPLE PREPARATION OF PLEURAL EFFUSIONS FOR CYTODIAGNOSTICS

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ABSTRACT

Pleural effusions – fluids that build up surrounding the lungs - can harbor malignant cells which are important to identify for diagnosis of cancer. However, rare malignant cells may lead to misleading diagnoses and a large background of leukocytes can prevent accurate identification of mutations of interest for targeted anti-cancer therapies. We developed a miniaturized microfluidic system that employs microscale vortices for the size-based isolation of malignant cancer cells and mesothelial cells. By processing mL volumes of pleural effusions and enriching target cells over a background of blood cells, we replace the traditional centrifugation step in the clinical lab. We demonstrate higher purity in 100% of the samples (n=30) where cells are made readily available for immunolabeling, standard cytology analysis and molecular analysis.

KEYWORDS

Laminar Microvortices, Liquid-Based Preparation, Inertial Microfluidics

INTRODUCTION

Pleural fluids abnormally accumulate in patients with cancer or other diseases and are extracted to determine the cause and to provide relief to the patient. Cytopathologists analyze pleural samples to determine the cause (presence of absence of cancer) by examining stained cell smears on a glass slide prepared via centrifugation and labeling [1]. However, blood cell components (leukocytes/erythrocytes) and other non-cellular materials in the sample create a background, making it difficult to locate potentially malignant epithelial cells of interest over a large field of view necessary for diagnostic accuracy. Harvesting large quantities of tumor cells in high purity could improve cytology-based diagnoses. We have previously demonstrated a miniaturized microfluidic system that recapitulates the high-throughput operations of enrichment and concentration of a standard laboratory centrifuge [2]. Here, we use the "Centrifuge Chip" for the isolation of larger cancer cells and mesothelial cells at high purity from complex biological fluids such as pleural effusions as a preparation step for analysis by traditional cytology. By processing a large volume of fluid and selectively enriching larger cells over a background of red and white blood cells we replace the traditional centrifugation step in the clinical lab while also potentially enabling more sensitive analysis of pure preparations originating from a larger volume.

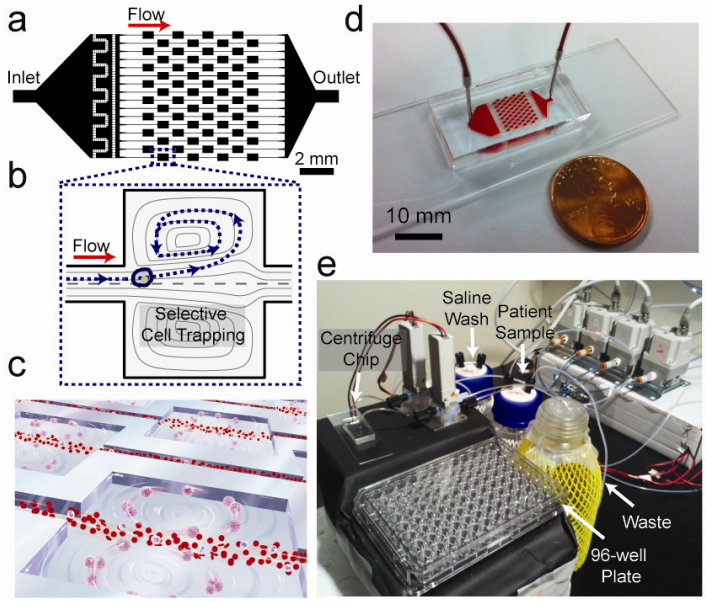

Figure 1: Device Design and Setup. (a,b) Schematic of selective cell trapping in a single microscale vortex and parallel device design. (c) Artist rendering of cancer cell capture from a bloody pleural sample (d,e) Centrifuge Chip device connected to an automated fluidic instrument to deliver patient pleural samples and saline wash through the Centrifuge Chip into the waste bottle. Trapped epithelial cells are made readily available 1) into a collection tube for further cytology slide comparisons with the original (Fig. 4) and/or 2) a well-plate for immunolabeling, imaging and analysis.

EXPERIMENT

The Centrifuge Chip approach employs unique inertial fluid physics to selectively collect larger cells in laminar fluid microvortices at high rates without clog-prone filters (Fig. 1a-c, 2) [2]. The device is connected to a custom-made automated fluid processing instrument to enrich larger cells from bloody pleural and peritoneal fluid samples (Fig. 1d,e). Purified cells are released into a small liquid volume and made readily available for i) immunolabeling, imaging and analysis (Fig. 3), ii) standard cytology slides (Fig. 4), and iii) molecular analysis. Figure 3 compares pleural fluid sample preparation conducted with a standard centrifuge vs. the Centrifuge Chip. Cells are immunostained with Cytokeratin-PE (epithelial cell), CD-45-FITC (leukocyte), and DAPI (nucleus). The Centrifuge Chip delivers a higher purity sample compared to the standard centrifuge.

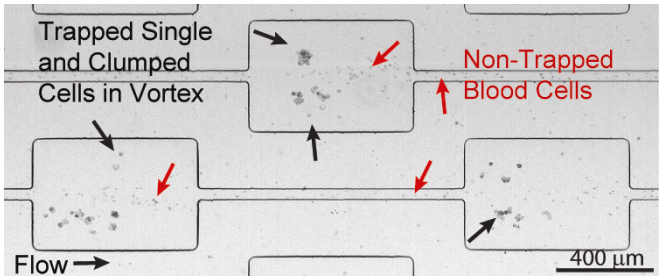

Figure 2: Processing of Pleural Sample in the Centrifuge Chip. Snapshot high-speed image of trapping of single and clumped large cells while smaller contaminating white and red blood cells are washed out.

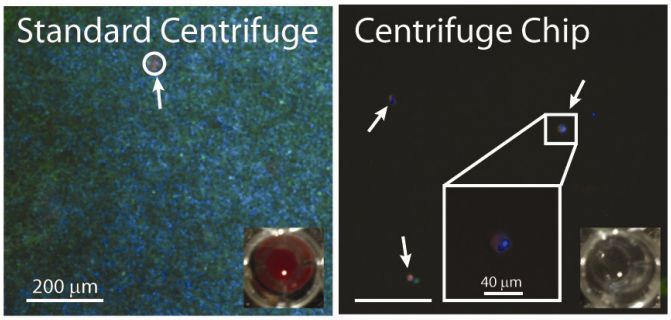

Figure 3: Purity Comparison of Patient Pleural Sample Processed with a Standard Centrifuge and Centrifuge Chip. Immuno- fluorescent images of CK+ (red), CD45+ (green), DAPI+ (blue). High purity after Centrifuge Chip processing leads to enhanced sensitivity for mutation or expression analysis. Insets show well-plate color (red and colorless). Arrows indicate CK+/DAPI+ epithelial cells.

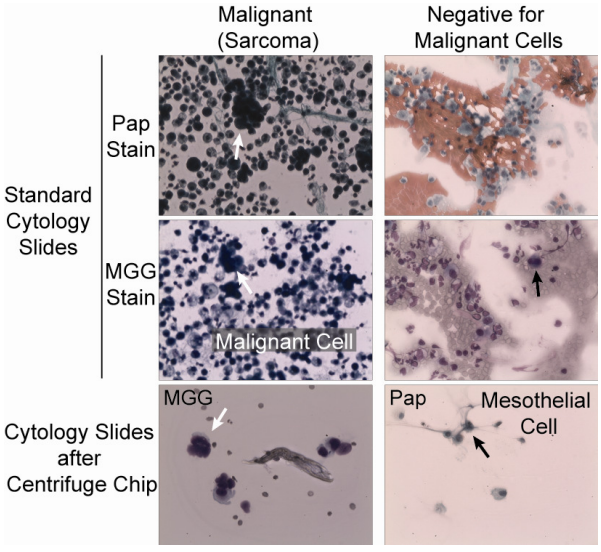

Figure 4: Reduced Background Cytology Slides When Prepared Using the Centrifuge Chip. Patient pleural samples with cytologist diagnosis using Papanicolaou (Pap) and May-Grunwald–Giemsa (MGG) stains. (Malignant) Malignant cells (white arrows) are found amongst a cellular background of immune cells in standard slides while little background is observed in the Centrifuge Chip slide. (Negative for Malignancy) Mesothelial cells (black arrows) found amongst a background of red and white blood cells in standard slides compared to Centrifuge Chip slide with reduced cellular background. Images obtained at 200x magnification.

RESULTS AND DISCUSSION

The Centrifuge Chip processes effusions at a flow rate of 6 mL/min from up to 50 mL liquid volume and concentrates larger cells (mesothelial and epithelial) (Fig. 2). Smaller leukocytes and erythrocytes are not stably trapped in vortices and are significantly reduced in the collected concentrated sample. Reduction in background and concentration from a large sample volume to a small field of view can aid rapid scanning and diagnosis by the cytopathologist (Fig. 3, 4).

Increasing the purity of the sample can also aid molecular diagnostics for targeted cancer therapies. Removing a large population of leukocytes that contain interfering wild type DNA can aid with the detection of genetic lesions that contribute to sensitivity or resistance to specific chemotherapies [3]. Thus, purity of the processed pleural sample becomes an important parameter for high sensitivity PCR-based mutational analysis, where previously a threshold of 40% purity was identified for high accuracy [3].

To quantify purity, unprocessed and Centrifuge Chip processed samples (n=30) were placed in a well-plate, immunostained, and analyzed for the presence of CK+/DAPI+ cells over the total number of cells (Fig. 5). Contaminating cells include white blood cells that stained positive for CD45, a leukocyte biomarker. Oftentimes, leukocytes become activated in acute or chronic inflammation cases and increase in cell sizes larger than 15 µm (the critical size necessary for cell trapping with the Centrifuge Chip). The Centrifuge Chip increased purity in all cases (30 of 30). The malignant cases (n=5) were diagnosed positive from cytological examination for adenocarcinoma (2 lung, 1 breast, and 1 gastric cancers) and rhabdomyosarcoma (alveolar) (Fig. 4 Malignant Case). Atypical cases (n=4) are typically diagnosed when suspicious cells are present and require further testing for final diagnosis. Most cases (n=21) were diagnosed negative for malignancy in addition to having acute or chronic inflammation, lymphocytosis, or reactive changes. Additionally, some atypical (2 of 4) and malignant cases (2 of 5) demonstrated purity greater than 40% (the threshold for accurate molecular analysis), suggesting it is possible for detecting specific gene mutations such as EGFR or KRAS. While some purity measurements do not exceed the 40%, higher purities can be achieved by increasing the critical size cutoff range of the Centrifuge Chip to eliminate leukocyte capture. High purity samples provided by a simple high-speed preparation step described here would enable pathologists and clinicians to explore and better proscribe targeted drug therapies with an easily accessed source of cells compared to solid tumor biopsies.

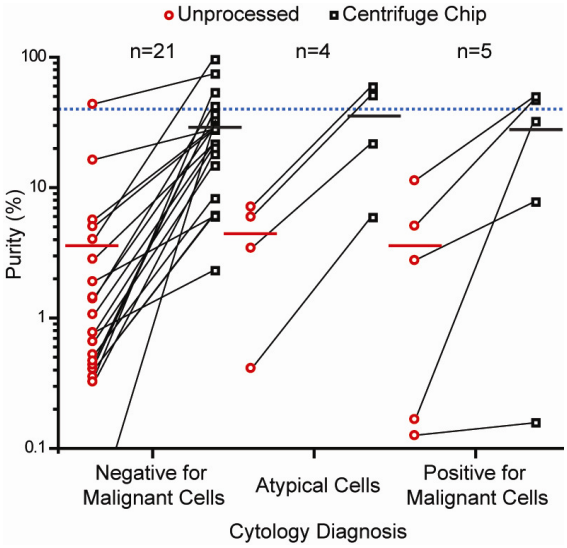

Figure 5: Purity Increases Upon Processing with the Centrifuge Chip. Purity is defined as the presence of CK+/DAPI+ cells over the total number of cells. All samples (n=30) demonstrate higher purity after processing with Centrifuge Chip. Solid lines indicate average of purity for respective diagnosis. Dashed line indicates 40% purity, a threshold previously proposed for accurate molecular analysis [3].

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