Detection of CTCs from Whole Blood of Lung Cancer Patients using Automated *Liquid Scan Microfluidic Chip Technology

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Abstract

Lung cancer is the most common cancer worldwide, causing ~1.6 million death every year. Detection of circulating tumor cells (CTC) in lung cancer patient blood is challenging due to the rarity and the complex analytical procedures involved in isolating them. The Liquid Scan™ system has been developed by Biofluidica to isolate CTCs with highly sophisticated microfluidic chip technology. The central technology of the Liquid Scan system is Biofluidica's programmable microfluidic chip that allows for the capture of CTCs in general as well as cells at the epithelial to mesenchymal transition (EMT).

We conducted a study of 23 staged lung cancer patients, in which patient blood was processed through the Liquid Scan system. Live CTCs bound to the chips were eluted and molecularly characterized thus indirectly characterizing the solid tumor(s). We were able to capture CTCs across all lung cancer stages (I, II, III, IV) using anti-EpCAM antibodies on the chip surface. CTCs were detected from Stage I patients using a minimal blood sample (1 ml) suggests Biofluidica's microfluidic chip technology can detect pre-symptomatic disease and early detection of residual disease or relapse.

Biofluidica's Liquid Scan automated platform will help to provide excellent patient care that delivers high quality data suitable to include clinical decisions as per cancer stage, response to treatment and early detection. Biofluidica's Liquid Scan can cut down the expensive and painful biopsy procedures generally used in the current health care system.

Table 1: Lung Cancer Patient Samples

Patient #	Type	Stage
1	Lung Adenocarcinoma	IV
2	Lung Adenocarcinoma	IV
3	Lung Adenocarcinoma	IV
4	NSCLC	IV
5	Lung Adenocarcinoma	IV
6	Lung Adenocarcinoma	III
7	Lung Squamous Cell Carcinoma	I
8	Lung Squamous Cell Carcinoma	IIB
9	Lung Carcinoma	IV
10	Lung Adenocarcinoma	IV
11	Lung Adenocarcinoma	IV
12	Lung Adenocarcinoma	IV
13	Lung Adenocarcinoma	IV
14	Lung Adenocarcinoma	IV
16	Lung Adenocarcinoma	II
17	Lung Adenocarcinoma	IB
18	Carcinoma	IV
19	Lung Adenocarcinoma	IIIA
20	Lung Cancer	I
21	Lung Squamous Cell Carcinoma	IV
22	Lung Adenocarcinoma	IV
23	Carcinoma	II

Table 1: Liquid Biospsy samples were obtained from lung cancer patients, presenting with lung adenocarcinoma, a form of non-small cell lung cancer (NSCLC).

Cell selection and release assay technology on microfluidic chip

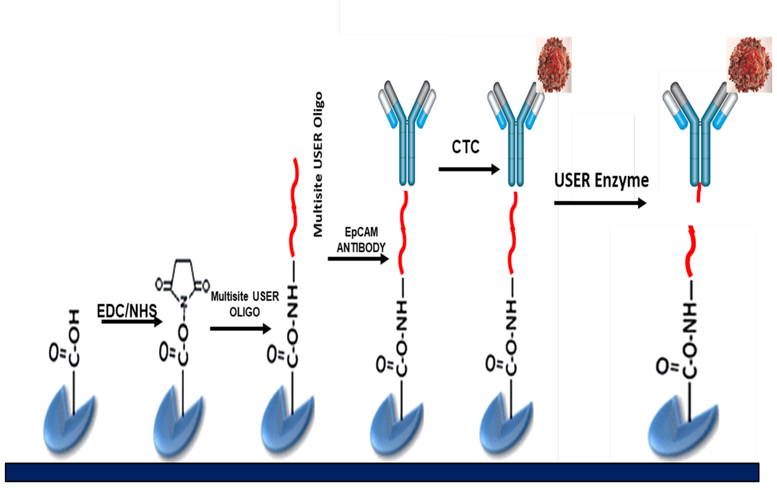
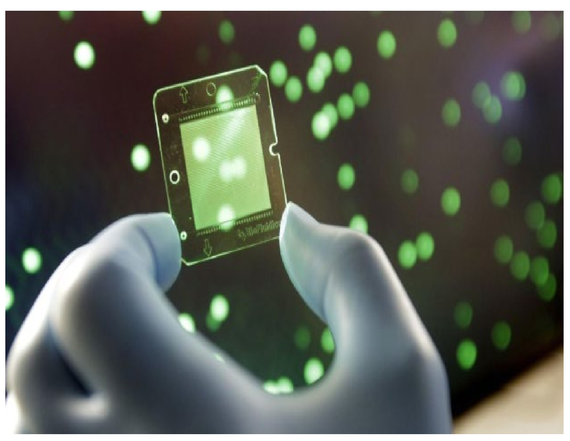


Figure 2. Ab tethered to surfaces by flexible proprietary linkers with features for targeted degradation are used for the positive selection of target cells. Incubation of the selected cells and linker with the USER™ Enzyme system removes the uracil residue and releases the selected CTC cells.

Methodology



System design to minimize biomarker loss. Many biomarkers are lost through sample handling from collection through processing. Three major sources for sample loss or alterations are: 1) Degradation, 2) Complex processing including sample prep 3) Interaction with instrument components. BioFluidica has developed a platform minimizing all sources of biomarker loss.

Whole Blood enters the instrument without preprocessing. The blood is directly introduced into the programable microfluidic chip.

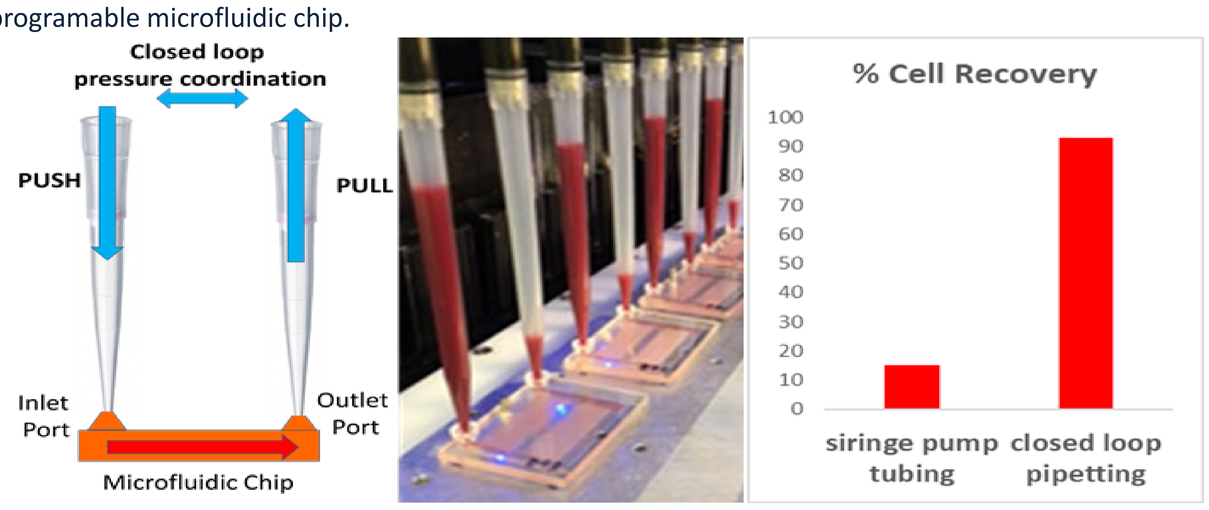


Figure 1a. The instrumentation does not use tubing, valves, or any other blood manipulation. Whole blood is introduced into the processing chip using **a** single step process: non-bind pipetting tip is used to aspirate the blood directly from the collection tube and to transfer it into the biomarker isolation chip. The same pipet is then used to push the blood through the isolation chip.

Minimizing Manipulation steps is important, our process requires no mixing of the blood, filtration, exposure to high pressure or rapid liquid movements on chip. We control the flow rate through extremely precise robotic software so that sensitive cells are never exposed to harsh and uncontrolled handling. Minimum exposure to sheer stress increases cell survival and improved cell recovery.

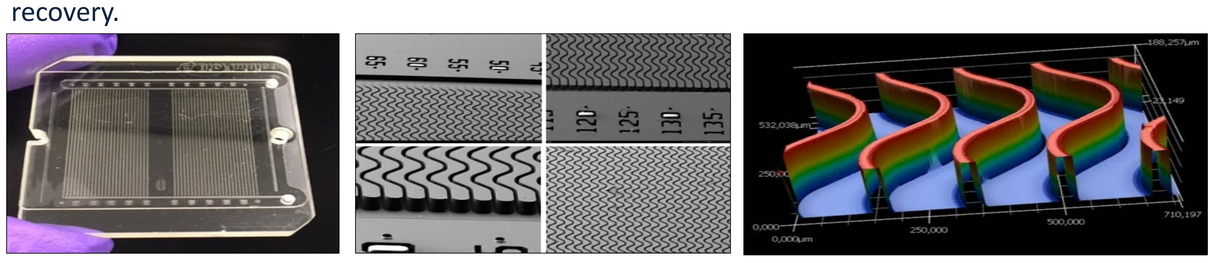


Figure 1b. Maximizing Capture Rates through high aspect ratio channel design with sinusoidal shape using parallel processing channel designs. The channel design maximizes the interaction of the cell biomarker with the capture channel surface, that is programable for different cell types, by providing the optimal roll rate along the channel wall. Even small changes in flow speed can impact the delicate cell capture efficiency either through increased cell lysis, interaction pressure with the capture wall surface and time of capture compound to the cell surface.

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Results

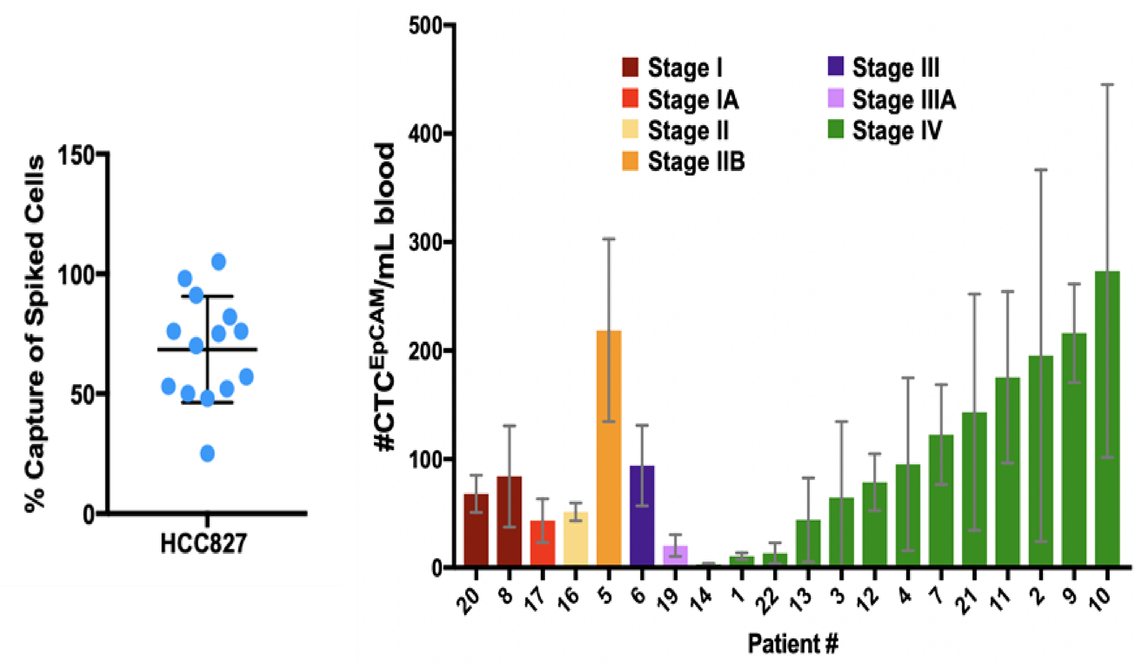

Figure 3. Liquid Scan Technology captures lung cancer CTCs. (A) *Demonstration of capture efficiency using CTC^{EpCAM} microfluidic device. Patient whole blood (1mL, n=14) was spiked with 100 pre-stained, EpCAM+ lung adenocarcinoma cells (HCC827), and capture efficiency of stained cells using the microfluidic device was measured. (B) CTCEpCAM cells were isolated from 1mL of patient blood using with the CTC^{EpCAM} microfluidic. Each patient sample was analyzed at least three times. Bars represent mean number of CTCs captured/mL of blood. Error bars represent standard deviation. Bar color corresponds to cancer stage according to the key.*

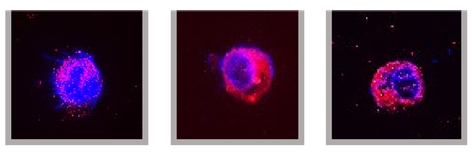

Figure 4. Cytologogical Analysis of Recovered Cells: CTCs confirmation from ICC staining using Ab EpCAM (red) and nuclear DNA stain by Hoechst (blue).

Conclusion

Capture of CTCs from all stages of lung cancer patients using EpCAM antibody on microfluidic chips speaks to the sensitivity and feasibility of Biofluidica's Liquid Scan technology. It highlight the superior performance of the Biofluidica's microfluidic chip approach relative to other competing technologies.

We demonstrate proof-of-concept for lung cancer applications, for which there currently exist no reliable CTC isolation and enrichment platforms with commercial potential.

The data also highlight the potential for (i) pre-symptomatic detection during the course of disease and (ii) early detection of residual disease relapse, two important clinical applications that would significantly improve patient care.

References

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