Automated Microfluidic Platform Allows for Interrogation of Tumor Biomarkers from Whole Blood

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Abstract

Blood contains a wealth of diverse tumor biomarkers, including circulating tumor cells (CTCs), extracellular vesicles (EVs), and cell free DNA (cfDNA). Biomarkers released from tumor cells harbor important signatures of disease including mutations, copy number variations, methylation changes, and/or chromosomal rearrangements. These biomarkers can be used to monitor disease progression or to tailor individualized treatment options in this era of personalized medicine. We describe an automated liquid biopsy platform, the Liquid Scan™, used to interrogate lung cancer patient blood for CTCs, cfDNA and EVs.

For CTC diagnostics, whole blood from patient samples was run through Biofluidica's customized chips coated with cancer specific antibodies. CTCs bound to the chips were eluted live and used for standard screening tests and molecular characterization of the tumor. We were able to capture CTCs from all stages (I, II, III, IV) of lung cancer patient blood samples using EpCAM antibodies coated on the chip surface. CTCs detected from Stage I patients using minimal blood samples (1 mL) suggests Biofluidica's microfluidic chip technology can detect pre-symptomatic disease and early detection of residual disease or relapse.

The Liquid Scan™ is also used for automated capture and isolation of cfDNA and EVs with different processing protocols, microfluidic chips, and reagents. Blood plasma is applied to the chip which contains 1.4 million diamond shaped posts. The specific biomarker is captured and released from the chip for analysis. Here we demonstrate the ability of the platform to efficiently capture cfDNA and EVs from cell lines and from patient samples with minimal background resulting in the ability to further analyze these biomarkers for genetic mutations. Current limitations of using cfDNA include the possibility of low abundance of ctDNA in blood and difficulty in isolating short fragment size populations of cfDNA (70-300bp). The Liquid Scan™ technology overcomes these limitations.

The different chip modifications coupled with Biofluidica's Liquid Scan™ automated platform allows for screening of different types of tumor biomarkers resulting in rapid processing from a single sample. This will help to provide excellent patient care that delivers high quality data suitable to assist in clinical decisions as per cancer stage, response to treatment and early detection. Biofluidica's Liquid Scan can reduce expensive and painful biopsy procedures generally used in the current health care system.

Methodology

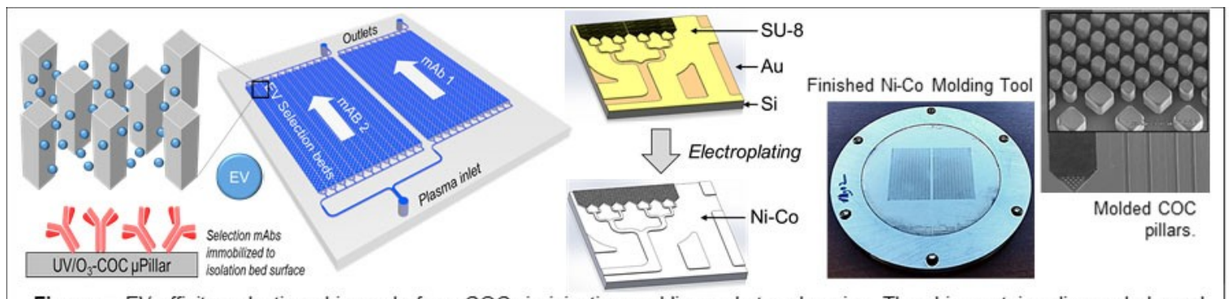

Figure EV affinity selection chip made from COC via injection molding or hot embossing. The chip contains diamond-shaped pillars that are activated using UV/O3 light with subsequent addition of mAb for selection of two types of EVs.

Figure 1. Maximizing Capture Rates through a bed designed using diamond shaped pillars. The bed design maximizes the interaction of the cell biomarker with the capture channel surface, which is programmable for different biomarker types, by providing the optimal flow to bind to diamond shaped pillars. Even small changes in flow speed can impact the delicate EV capture efficiency either through increased lysis, interaction pressure with the capture wall surface and time of capture compound to the biomarker surface.

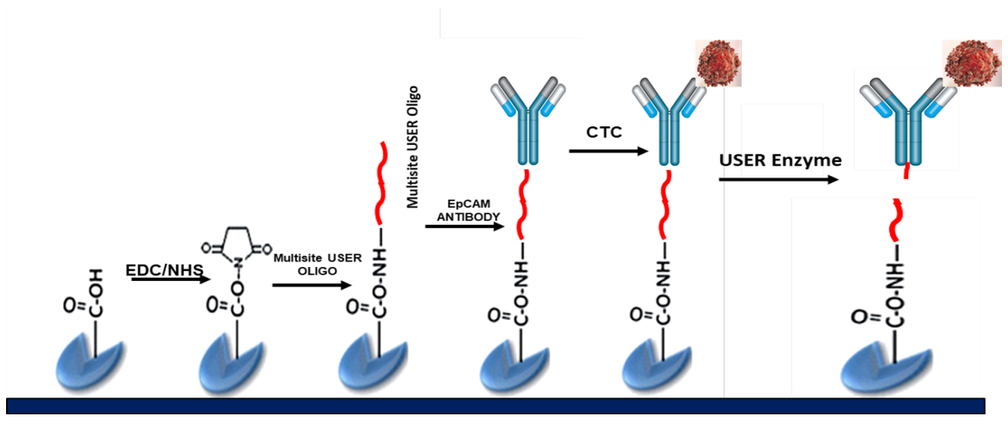

Figure 2. Cell selection and release assay technology on the microfluidic chip Antibody tethered to surfaces by flexible proprietary linkers with features for targeted degradation are used for the positive selection of target cells. Incubation of the selected cells and a linker with the USER™ Enzyme system removes the uracil residue and releases the selected CTCs.

Results

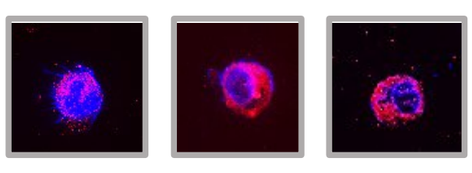

Figure 3. Cytological Analysis of Recovered Cells: Double staining using an EpCAM antibody (red) and nuclear DNA stain by Hoechst (blue) confirms specific capture of CTCs using the Liquid Scan™ technology.

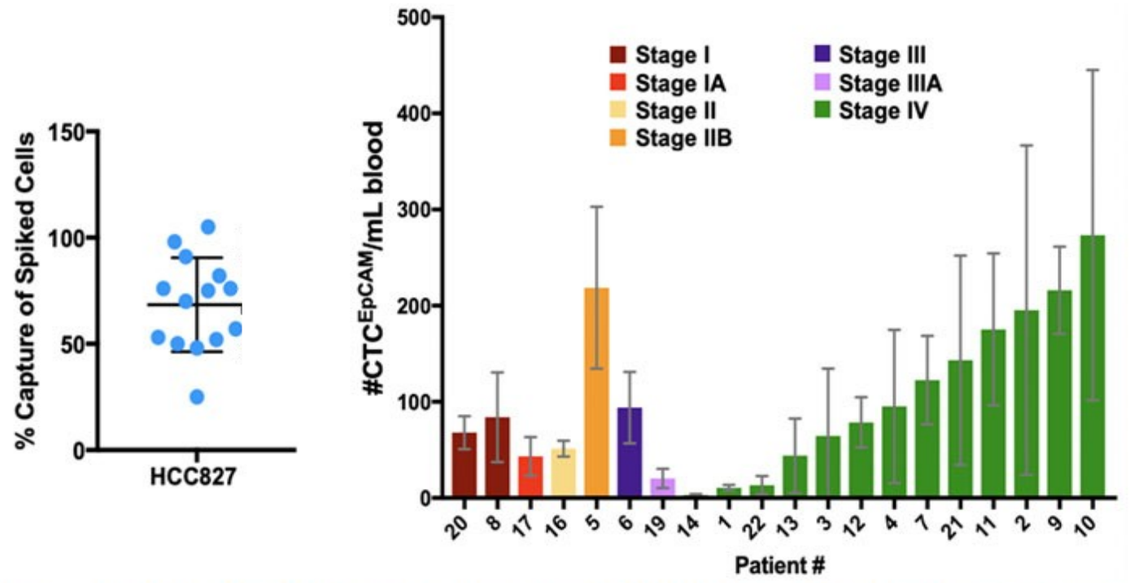

Figure 4. Liquid Scan™ Technology captures lung cancer CTCs. A) Demonstration of capture efficiency using CTC EpCAM microfluidic device. Patient whole blood (1mL, n=22) was spiked with 100 pre-stained, EpCAM+ lung adenocarcinoma cells (HCC827), and capture efficiency of stained cells using the microfluidic chip was measured. (b) CTC EpCAM cells were isolated from Liquid Biopsy samples obtained from lung cancer patients, presenting with lung adenocarcinoma, a form of non-small cell lung cancer (NSCLC). 1 ml of patient blood using the CTC EpCAM microfluidic chip was processed. 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.

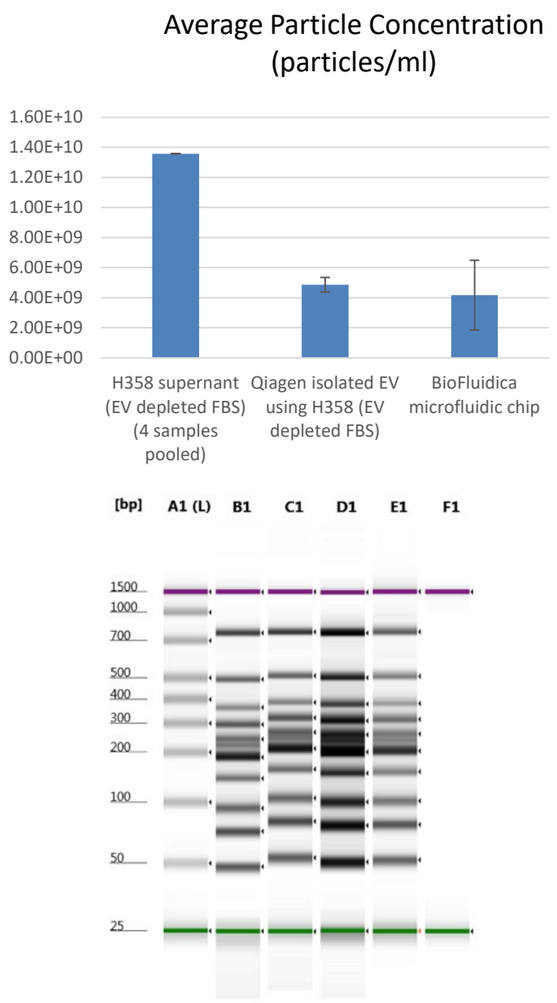

Figure 5. Extracellular vesicle capture from H358 cell line media supernatant. Particle concentration was determined using Nanoparticle Tracking Analysis. Supernatant from media collected from H358 cell lines were isolated using the Qiagen exoEasy Maxi kit and utilizing the BioFluidica Liquid Scan automated microfluidic platform. Using the Liquid Scan isolation of the EVs could be done entirely on the automated platform without user manipulation and in a comparable amount of time to the Qiagen exoEasy kit.

Figure 6. BioFluidica cfDNA chips capture low molecular weight DNA. As proof of concept, low molecular weight (LMW) DNA ladder using various conditions was run through the cfDNA microfluidic chip and eluted following isolation. LMW DNA was recovered demonstrating the efficacy of the Liquid Scan to isolate cfDNA.

A1: High Sensitivity D1000 ladder
B1: LMW ladder, condition 1
C1: LMW ladder, condition 2
D1: R5, repeat of C1
E1: R6, repeat of C1
F1: nuclease free water

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. 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.

We demonstrate proof-of-concept for lung cancer applications, for which there currently exist no reliable CTC isolation and enrichment platforms with commercial potential. We also demonstrate the ability of the liquid scan platform to isolate EVs from the lung cancer cell line H358 and the capability to capture Low molecular weight DNA.

Biofluidica's Liquid Scan™ automated platform allows for screening of different types of tumor biomarkers resulting in rapid processing from a single sample.

References

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