Isolation of cancer cell-derived extracellular vesicles utilizing an automated microfluidic platform

Jerry Lu, Ph.D.,Mateusz Hupert, Ph.D., Mengjia Hu, Ph.D., Paul Diaz, Ph.D., Alena Bartakova, M.D., Jennifer Barber-Singh, Ph.D., Veronica Cheung, Sangeetha Purushotham, Ph.D., Steven Soper, Ph.D., Judy Muller-Cohn, Ph.D., & Rolf Muller, Ph.D.

Abstract

Once dismissed as carriers of inconsequential cellular debris, the importance of extracellular vesicles (EVs) as mediators of tumor progression and metastasis is now widely recognized[1]. Extracellular vesicles (EVs) can carry proteins, metabolites, and nucleic acids from their cell of origin to a recipient cell[2,3]. Consequently, they are important regulators of cell-to-cell communication. EVs from cancerous cells can subsequently promote survival and growth of primary tumors and metastases[4-6]. EV contents, at least in part, resemble that of their parental cell. Thus, the biomarkers carried within EVs can be used for detection and genetic analysis of many types of disease, including cancer. EVs are also stable and present in most bodily fluids including blood[7-10], therefore EV-based liquid biopsy analysis can be a convenient, minimally invasive method for disease diagnosis, monitoring, and predictive tracking.

BioFluidica, Inc. has developed a fully automated microfluidic platform to isolate multiple biomarkers including CTCs, cfDNA and EVs. Our microfluidic devices are injection molded from common plastics and are scalable to up to a million devices per year. Whole blood or plasma is applied to the microfluidic device which contains 1.4 million diamond shaped posts spaced 5 microns apart utilizing our fully automated Liquid Scan platform. Here we demonstrate the ability of the Liquid Scan automated platform to efficiently capture EVs from cell lines and plasma from patient samples with minimal background and the ability to further analyze these biomarkers for genetic mutations.

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 or plasma enters the instrument without preprocessing. The blood is directly introduced into the programable microfluidic chip.

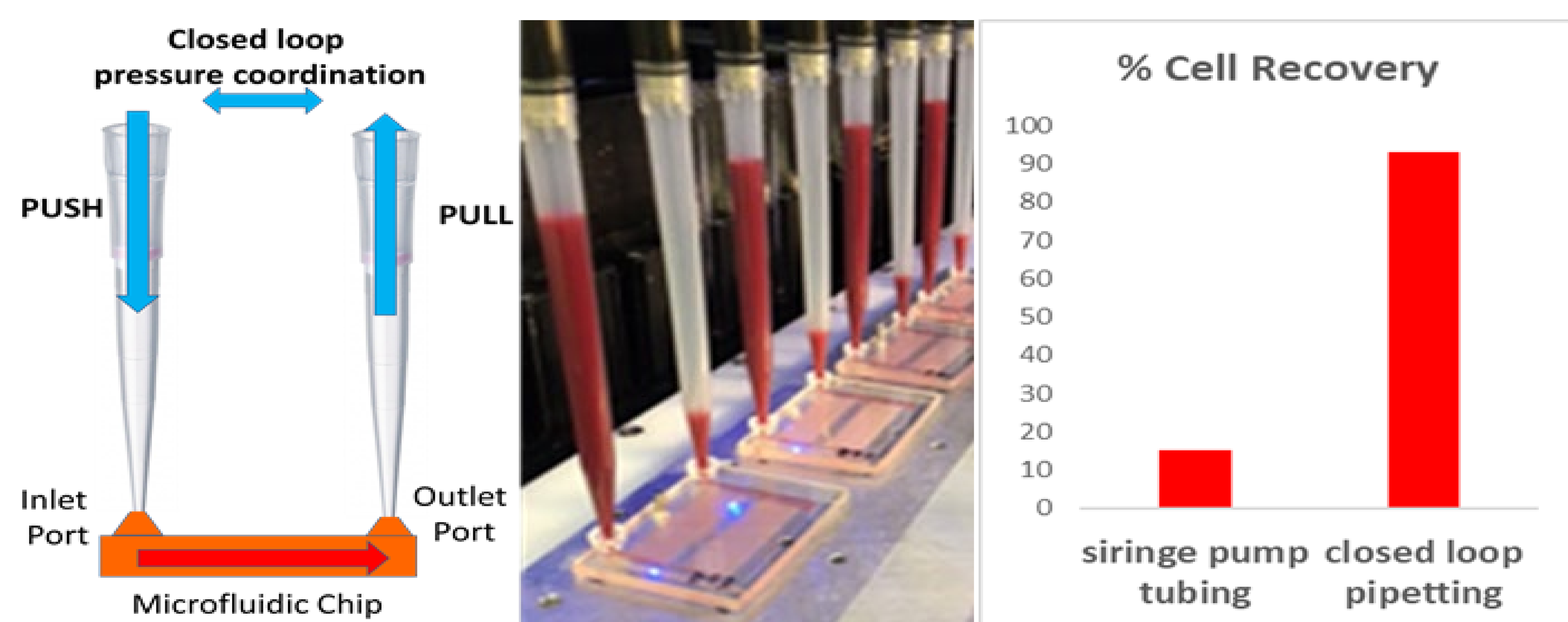

Figure 1. The instrumentation does not use tubing, valves, or any other blood manipulation. Whole blood is introduced into the processing chip using **a** single step process: non-bind pipetting tip is used to aspirate the blood directly from the collection tube and to transfer it into the biomarker 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 biomarkers are never exposed to harsh and uncontrolled handling. Minimum exposure to sheer stress leads to improved recovery.

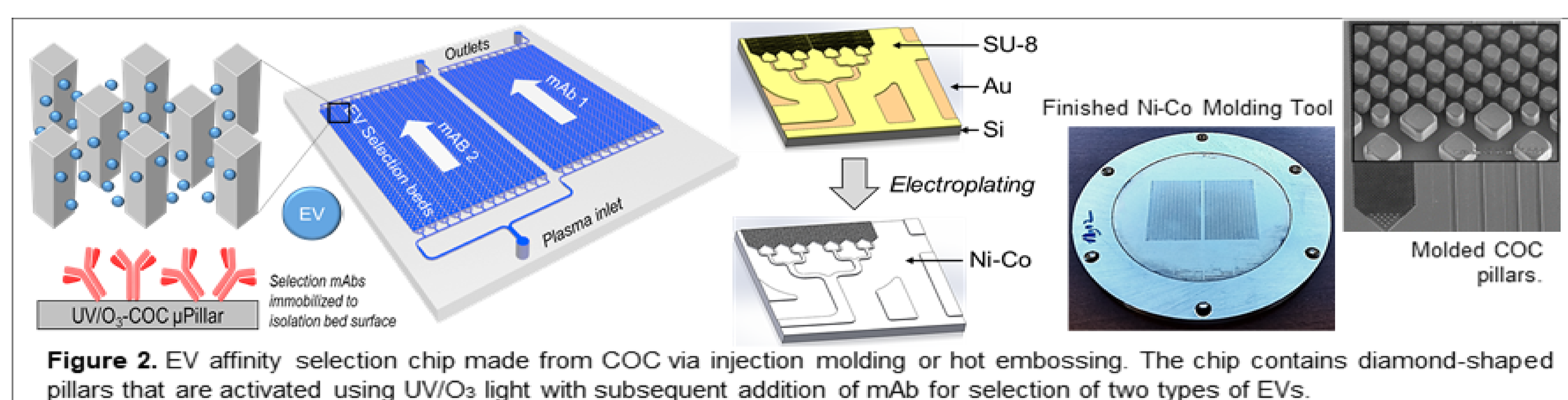

Figure 2. EV affinity selection chip made from COC via injection molding or hot embossing. The chip contains diamond-shaped pillars that are activated using UV/O3 light with subsequent addition of mAb for selection of two types of EVs.

Figure 2. 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, that 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.

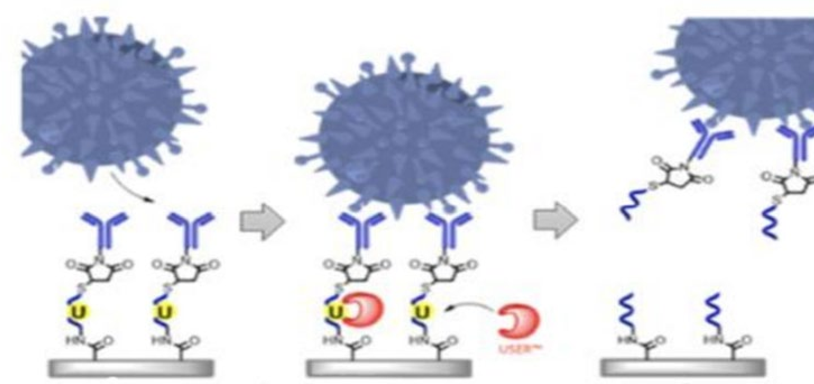

Figure 3. EV selection and release assay. Ab immobilized to surfaces using oligonucleotide linkers containing a uracil residue are used for the positive selection to target biomarkers. Incubation of the selected biomarker and ssDNA linker with the USER™ Enzyme system removes the uracil residue and releases the selected biomarker.

Results

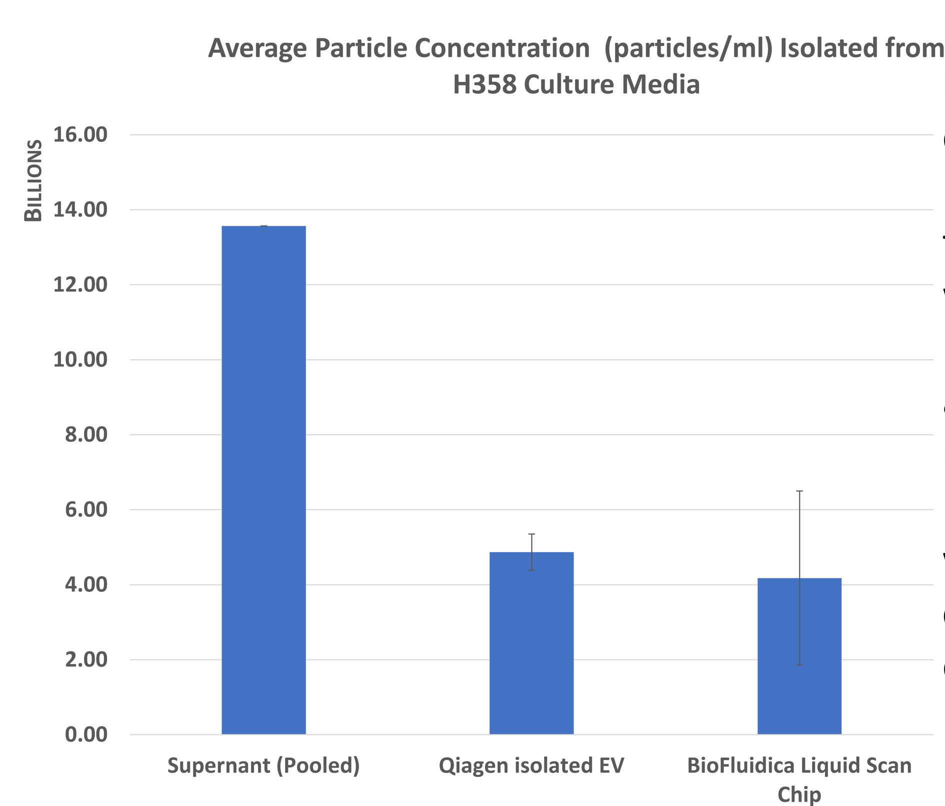

Figure 4. Extracellular vesicle capture from H358 cell line media supernatant. Particle concentration was determined using Nanoparticle Tracking Analysis. Supernatant from media collected from H358 cell lines were isolated using the Qiagen exoEasy Maxi kit and utilizing the BioFluidica Liquid Scan automated microfluidic platform. Using the Liquid Scan platform, 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.

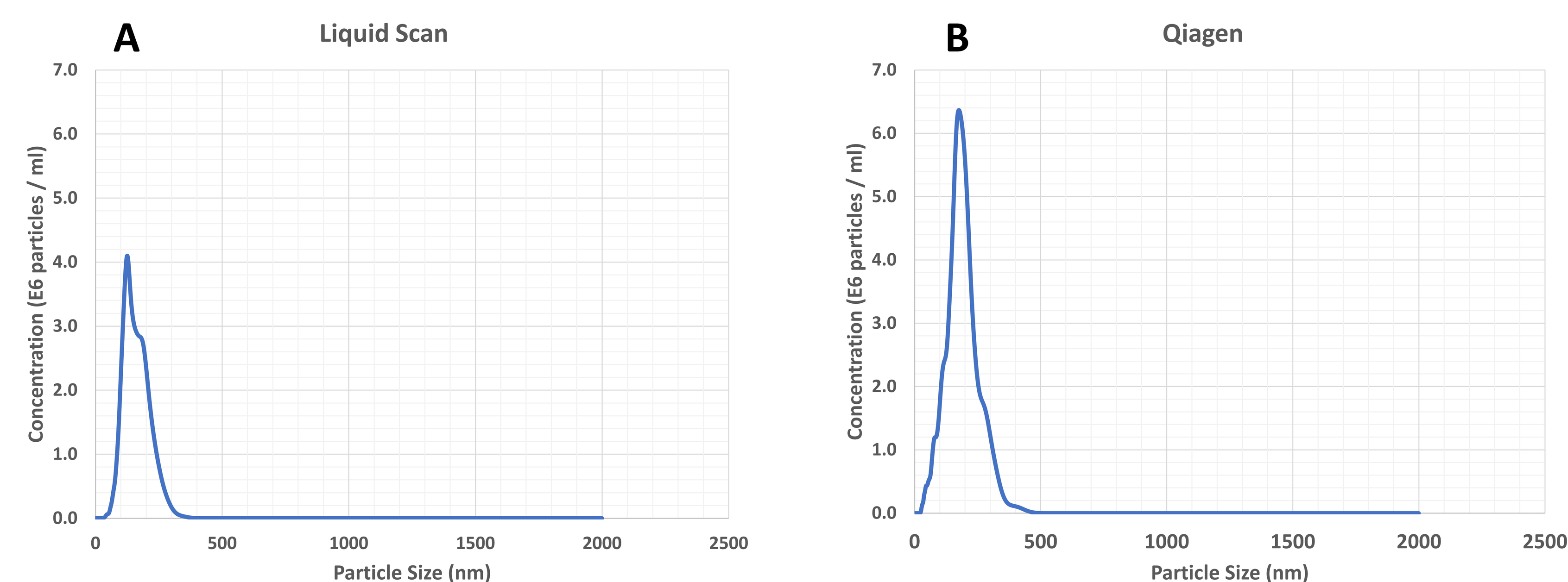

Figure 5. Size distribution of EVs isolated by the Liquid Scan platform (**A**) or the Qiagen exoEasy kit (**B**) Average particle size utilizing the Liquid Scan platform is 169nm. Using the Qiagen exoEasy kit, the average particle size is 182nm. EV's were isolated from 1.0 ml and 30 ml of H358 supernatant via the Biofluidica Liquid Scan platform or the QIAgen exoEasy Kit, respectively.

Conclusion

BioFluidica's automated microfluidic approach enables capturing EVs on a comparable scale to Qiagen's exoEasy kit. We will evaluate and optimize the steps for EV isolation in human plasma to increase capture efficiency and decrease the isolation time and then proceed to downstream analysis of RNA isolated from the EVs to demonstrate pre-clinical utility with a high recovery rate and purity. Our long term goal is the development of a fully integrated and automated microfluidic platform for the enrichment of EVs with high recovery and reproducibility. The platform will provide high specificity and throughput, and be cost effective as a commercially viable technology.

References

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