ApoStream™, a Novel Device for Antibody-Independent Capture of Circulating Tumor Cells from Blood of Patients with Various Types of Cancer

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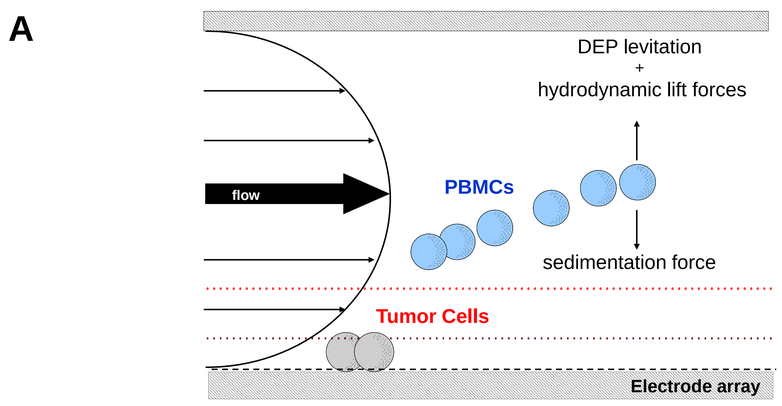
Abstract

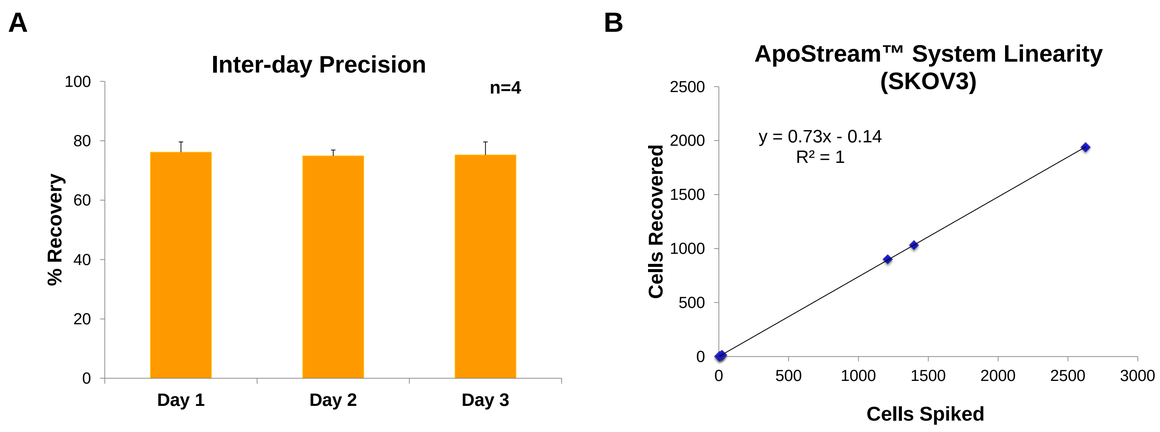
Background: The detection of circulating tumor cells (CTCs) using immunomagnetic EpCAM-based capture methods has been conceptually accepted as a “liquid tumor biopsy”. However, these methods have limited the recovery of CTCs for molecular profiling applications. A novel continuous flow dielectrophoresis field-flow fractionation (DEP-FFF) device, ApoStream™, was developed for antibody-independent capture of CTCs, with improved recovery across multiple cancer types and preserved viability of CTCs for downstream characterization. **Methods:** The performance of ApoStream™ was demonstrated using a low EpCAM expressing cell line, SKOV3, spiked into blood. ApoStream™ was also used to capture CTCs from cancer patient blood. Prostate, breast and NSCLC CTCs were stained for cytokeratin (CK), CD45, and DAPI; melanoma CTCs were stained with S100, CD45 and DAPI. CTC enumeration was performed using laser scanning cytometry. Trypan blue exclusion was used to determine cell viability after ApoStream™ separation. **Results:** In system precision performance studies, average inter-day recovery on the ApoStream™ was 80.3 3.5%, CV = 4.3% when cancer cells were spiked into buffer, and 78.5 3.0%, CV = 3.3% when cancer cells were spiked into ~10 million healthy peripheral blood mononuclear cells (PBMCs). Linearity performance was demonstrated by spiking 5 – 2600 SKOV3 cells into ~10 million PBMCs (R^2=1). Cell viability was not affected by processing through the ApoStream™ device. High CTC recovery from metastatic cancer patient blood samples was obtained with counts ranging from 0 – 2630 (NSCLC, n=66), 0 – 3490 (prostate, n=29), 10 – 968 (breast, n=11), and 3 – 3120 (melanoma, n=13) CTCs per 7.5mL blood. Positive CTC counts were obtained in 90% of NSCLC samples, 93% of prostate cancer samples, 100% breast cancer and melanoma specimens. There were no CK+ cells detected in healthy donor blood controls. **Conclusions:** Improved CTC recovery from various cancer types was demonstrated with the ApoStream™ device. ApoStream™ provides an antibody-independent method for capture of viable CTCs that enables further downstream molecular characterization of rare cells for use in clinical applications.

ApoStream™ System



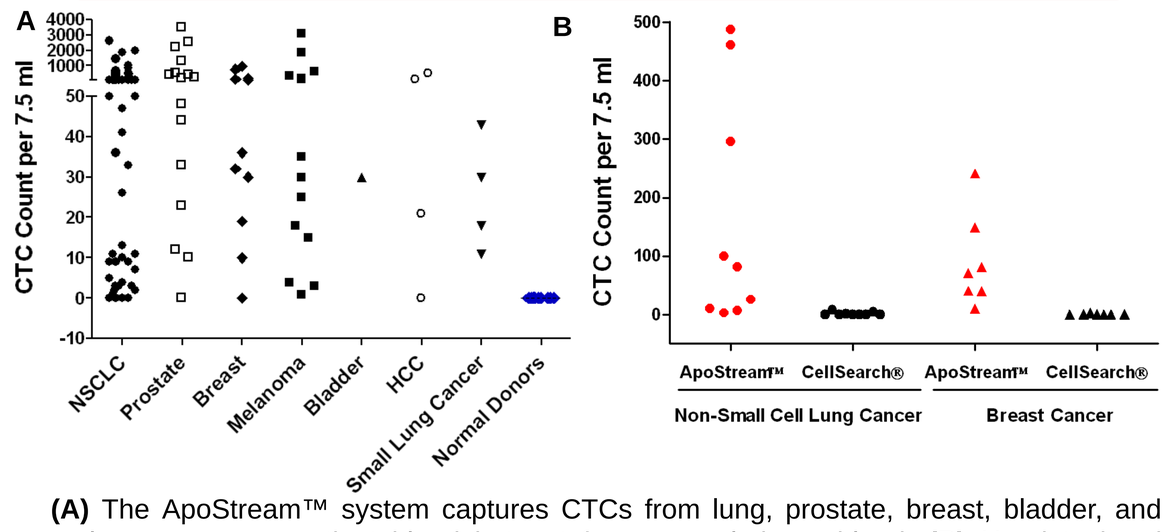
(A) Dielectrophoretic, hydrodynamic and sedimentation forces are balanced to attract CTCs and repel normal cells from the chamber floor. CTCs are collected through a port located in the chamber floor while normal cells flow into a waste port. **(B)** Cross-over frequencies from over 60 different tumor cell types including breast, colon, ovarian, lung and melanoma cell lines and from peripheral blood mononuclear cells (PBMCs) were determined. The differences in cross-over frequencies between cancer and normal cells enable ApoStream™ to separate CTCs from normal cells.

ApoStream™ Performance



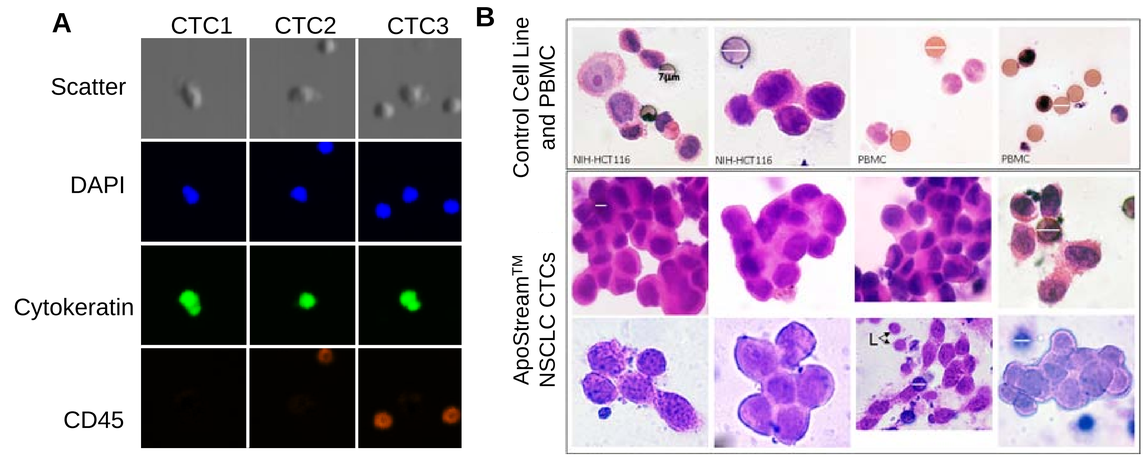
(A) Recovery of SKOV3 cancer cells spiked into PBMCs shows inter-day precision of 78.5 3.0%, CV = 3.3%. **(B)** Device linearity (R^2 = 1.0) was demonstrated by spiking 4 to ~2600 SKOV3 cells into ~10 million PBMCs from 7.5 mL normal human donor blood.

High CTC Recovery Using ApoStream™



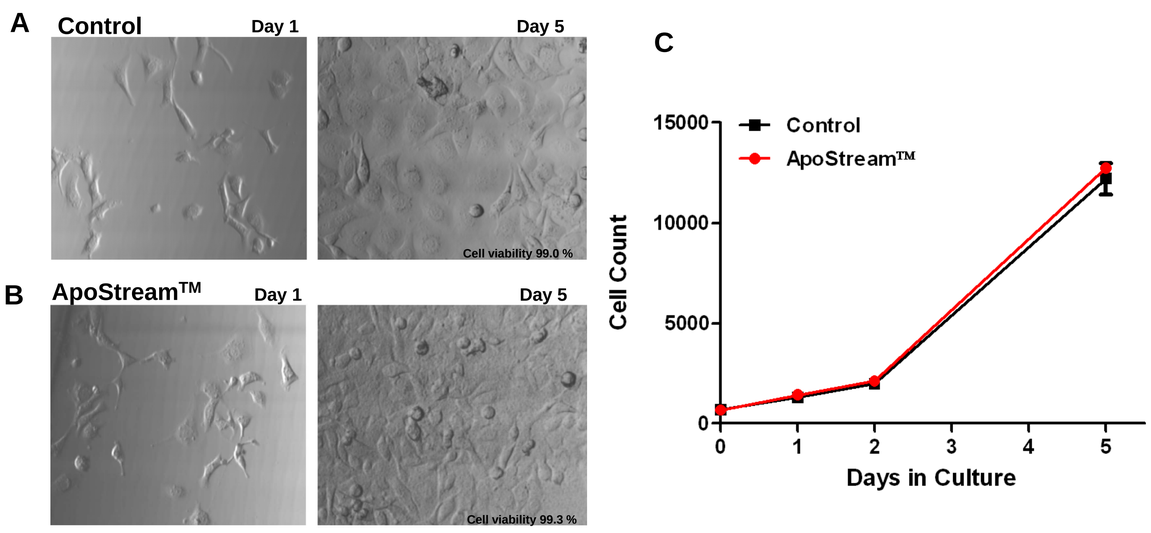
(A) The ApoStream™ system captures CTCs from lung, prostate, breast, bladder, and melanoma cancer patient blood but not from normal donor blood. **(B)** Head to head comparison between ApoStream™ and CellSearch® shows greater recovery of CTCs from non-small cell lung cancer (NSCLC) and breast cancer patient blood samples.

LSC and H&E Analysis of CTCs



(A) CTCs from NSCLC patients captured by ApoStream™ were identified by immunofluorescent staining using standard DAPI+/CK+/CD45- phenotype. **(B)** H&E staining of CTC clusters isolated from the blood of NSCLC patients.

ApoStream™ Preserves Cell Viability



(A) Images of SKOV3 cell proliferation (Control = no ApoStream™). **(B)** Images of SKOV3 cell proliferation after ApoStream™ separation from PBMCs. **(C)** Control cells and ApoStream™ recovered SKOV3 cancer cells show exponential growth.

Conclusions

- ApoStream™ separation exploits the differences in dielectrophoretic properties between cancer cells and normal cells.
- ApoStream™ recovery performance is robust.
- ApoStream™ is antibody-independent and captures CTCs from various types and stages of cancer.
- ApoStream™ captured higher numbers of CTCs from NSCLC and breast cancer patient blood compared to the EpCAM dependent CellSearch® method.
- ApoStream™ preserves cell viability enabling genetic and molecular characterization.

Acknowledgments

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