Novel Herringbone Chip Design for the Isolation of Circulating Prostate and Breast Cancer Cells from Liquid Biopsies Using the CD71 Receptor

Bhagya Wickramaratne and Dimitri Pappas*
Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, TX, 79409

Introduction

➢ Cancer

Cancer is among the leading causes of death worldwide. Over 100 billion dollars was spent in cancer related care in the United States only, in 2017.

➢ Types

- Hematopoietic or non-hematopoietic origin.
- Prostate cancer is the 5th leading cause of death in men.
- Invasive breast cancer is the most common type of cancer affecting women.

Approaches

➢ Current Screening Techniques

Prostate Cancer	Breast Cancer
• Digital Rectal Examination	• Mammogram
• Serum PSA level	• MRI Screening

Require prior knowledge of cancer type.

➢ Treatment Options

- Chemotherapy
- Immunotherapy
- Stem cell transplant (SCT)

➢ Point-of-Care Tests

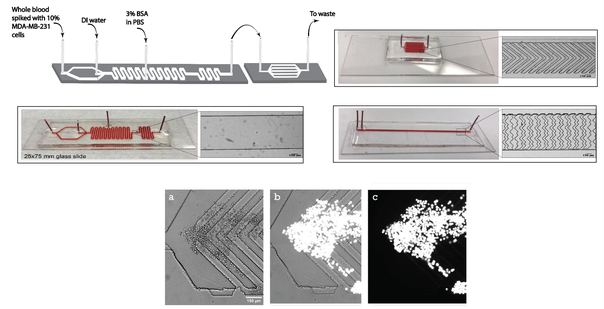
- "Sample in, answer out" devices.
- Small sample volumes.
- Sensitivity comparable to clinical standards.
- Deterministic lateral displacement, inertial and Dean flow methods, and affinity ligand separations.

Flow cytometry, using the EpCAM antigen is the gold standard for detecting circulating tumor cells (CTCs) from liquid biopsies. However, EpCAM is not expressed in CTCs of mesenchymal origin, cells having undergone the epithelial-to-mesenchymal transition, and leukemic cells.

Methods

➢ Microfluidic separation

In a blood, proliferating tumor cells have higher CD71 expression than mature leukocytes. This difference in expression can be exploited for affinity cell separations.



Microscope images of captured MDA-MB-231 cells (a) white light (b) white and fluorescent light (c) fluorescent light

Results

➢ Capture metrics

$$Enrichment\ factor = \frac{Purity\ of\ captured\ cells}{Purity\ of\ initial\ sample}$$

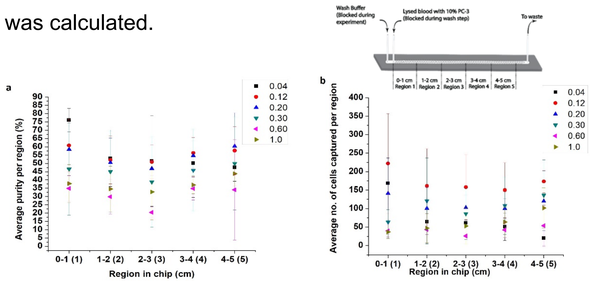
$$Purity = \frac{N_{target}}{N_{total}} \times 100$$

Experiment		Cancer cells		Purity of captured cells		Enrichment	
Method no.	Spike %	Concentration in blood (cells/mL)	Standard deviation	%	Standard deviation	Enrichment factor	Standard deviation
2 cm single-channel chip PC-3 cells| 0.04 mL/hr	20	336	133	94	7	5	0.4
	10	133	7	64	22	6	2
	1	17	1	56	22	56	22
	0.5	8	1	12	12	24	24
	0.1	3	0.02	6	4	46	32
2 cm single-channel chip PC-3 cells| 0.12 mL/hr	0.5	8	0.1	25	6	49	13
	0.1	1	0.2	2	1	21	9
5-channel chip PC-3 cells| 0.2 mL/hr	0.1	2	0	34	5	339	48
5-channel chip MDA-MB-231 cells| 0.2 mL/hr	10	176	10	93	6	9	1
	1	17	1	72	23	72	23
	0.5	7	1	63	30	125	60
	0.1	2	0	43	35	432	352

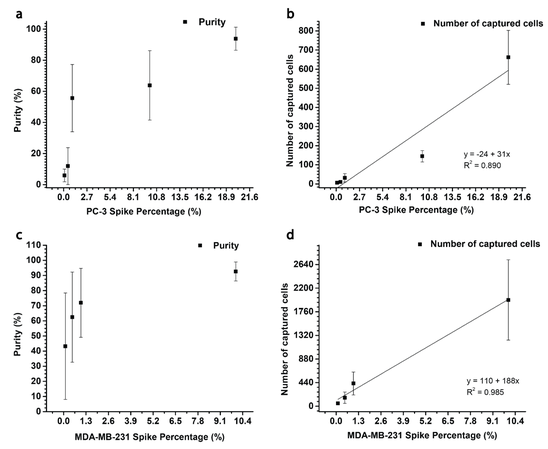
Results

➢ Determining Flow Rate and Capture Regions

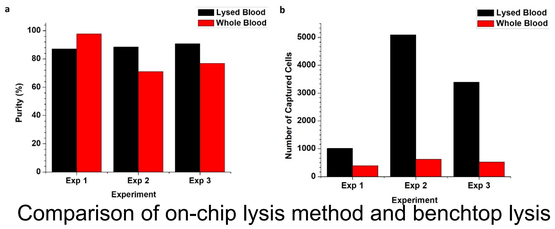
10% PC-3 spiked lysed blood and flowed through a straight channel at flow rates ranging from 0.04 to 0.12 mL/hr. The number of cells captured and the purity of each chip region was calculated.



➢ Limit of Detection



Graphs a-d show the purity and number of cells captured from the concentration studies for PC-3 and MDA-MB-231. LOD was 1 cell/μL concentration. At a 0.1% spike to lysed blood, PC-3 cells were captured at 34 ±5% purity and MDA-MB-231 cells at 43 ±35% purity.



Comparison of on-chip lysis method and benchtop lysis method using a 10% spike of MDA-MB-231 cells.

Conclusion

- The 5-channel herringbone chip has reduced the detection limit by two orders of magnitude.
- The 5-channel chip needs to allow a higher throughput to detect cells rarer than 1 cell/μL.
- The on-chip lysis method can successfully separate higher spike concentrations (>10%) but needs improvement for lower spikes concentrations.

Future Directions

The 5-channel chip demonstrates that parallel flow can increases the probability of detecting rare cells.

- Pan-cancer screening by unspecialized personnel.
- Captured cells can be used for seeding.
- Personalized drug testing.
- Multi-parameter testing on parallel channels (e.g. Sepsis)

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References

1. Lyons, V. J.; Helms, A.; Pappas, D., The Effect of Protein Expression on Cancer Cell Capture using the Human Transferrin Receptor (CD71) as an Affinity Ligand. *Analytica Chimica Acta* **2019**.
2. Gayet, M.; van der Aa, A.; Beerlage, H. P.; Schrier, B. P.; Mulders, P. F.; Wijkstra, H., The value of magnetic resonance imaging and ultrasonography (MRI/US)-fusion biopsy platforms in prostate cancer detection: a systematic review. *BJU international* **2016,** *117* (3), 392-400.
3. Senkus, E.; Kyriakides, S.; Ohno, S.; Penault-Llorca, F.; Poortmans, P.; Rutgers, E.; Zackrisson, S.; Cardoso, F.; Committee, o. b. o. t. E. G., Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up†. *Annals of Oncology* **2015,** *26* (suppl_5), v8-v30.
4. Wickramaratne, B.; Ivey, M.; Pappas, D., Isolation of proliferating cells from whole blood using Human Transferrin Receptor in a two-stage separation system. *Talanta* **2019**, 731-738.