

Affinity and Size Capture of Circulating Tumor Cells: A Platform for Increased Sensitivity

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Introduction

Cancer is a disease of uncontrolled cell growth and dissemination. As tumors increase in size and vascularity, populations of cells break off from the primary tumor, enter into the circulation, and are transported to distant parts of the host. These circulating tumor cells (CTCs) are subject to immunological surveillance, clearance by the liver, and shear forces of turbulent blood flow. Each of these factors contributes to cellular damage and invariably leads to rapid removal of the cells from the circulation. It is thought that the primary tumor continuously releases huge numbers of cells that immediately replenish the loss, resulting in a steady state of cells in the circulation.

Microfluidic technology has advanced the field of CTC capture^{1,2}. One of the most common methodologies is based on affinity capture, where specific, immobilized antibodies on a chip bind to cell surface antigens and trap the cells. A second technique employs size filtration. CTCs are usually larger than normal leukocytes and this size differential can be exploited to enrich CTCs from their smaller counterparts.

In this study, we now describe a new CTC platform from On-Q-ity that combines both affinity and size capture. This dual capture technology has the potential for more efficient CTC capture than either technology alone. Moreover, because the cells remain viable the On-Q-ity platform, it will provide an easier path toward more sophisticated molecular characterization of these CTCs, ultimately leading to more effective management of disease.

Methods

Cell lines

The non-small cell lung cancer cell line H1650 and the breast cancer cell lines SKBR-3 and MDA-MB-231 were all purchased from ATCC (Manassas, VA). Cells were cultured in RPMI media supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and harvested non-enzymatically at 50-75% confluence. They were then resuspended in PBS, counted and spiked into whole blood for analysis.

Blood samples

Whole, EDTA blood from healthy donors was obtained from Research Blood Components (Brighton, MA). Following standard venipuncture into purple-top tubes, the blood was transported to our facility on cold packs. Blood was stored at RT overnight before use to mimic aged blood received from locations requiring overnight shipment.

Chips

The two chip models, On-Q-ity T7 and On-Q-ity C5, were fully assembled at On-Q-ity. Plastic slides were embossed using a silicone mold to produce an open chamber containing multiple posts. For the T7 chip, a series of posts with a fixed diameter were arranged in a geometric pattern and separated by a constant gap size. An offset

in spacing every few rows generates pinch points which redirect sample flow. The On-Q-ity C5 chip is composed of multiple posts arranged in a gradient pattern, with constant post diameters and gap distances within a sector. Decreasing post diameters and gap sizes from sector to sector across the length of the chip more efficiently redirect flow than in the On-Q-ity T7 chip. Schematic diagrams of the two chip types are shown in Fig. 1.

Fig. 1a – On-Q-ity T7 chip

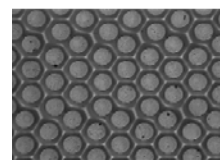
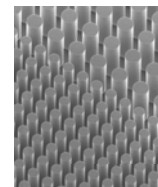


Fig. 1b – Gradient On-Q-ity C5 chip



Surface chemistry

A mouse, anti-EpCAM capture antibody was conjugated to the plastic chip surface through a Dextran linkage. Briefly, the plastic was first activated by oxidation and followed by covalent Dextran binding through an amino-hydroxyl bond. Antibody was then bound to the Dextran followed by reduction of the unreactive sites (Fig. 2). Unbound reagents were washed away and a cover was added, creating a liquid-tight sealed chamber. These biochemically “functionalized” chips were then assembled with tubing connected to inlet and outlet ports and stored at 4° C until use. A fully assembled chip is shown in Fig 3.

Fig. 2 - Dextran chemistry

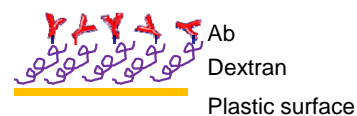


Fig. 3 - Fully assembled chip



Blood processing

Blood, without any pre-treatment, was passed through the chip from large to small gap size, at a constant flow rate directed by a peristaltic pump (Fig. 4). Captured cells on the chip were then fixed, permeabilized, and stained with a pan-cytokeratin PE-MAb, an anti-CD 45 FITC-Ab, and DAPI for fluorescent visualization. All reagents were then washed out, leaving the chip filled with PBS. The tubing was removed and ports sealed in preparation for imaging.

Fig. 4 - Blood processing station



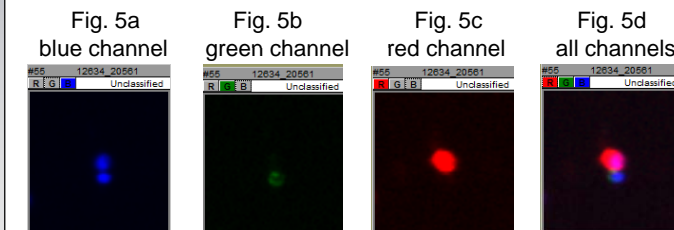
Imaging

Chips were imaged automatically under 5x magnification using a fluorescent microscope system. CTCs were identified by blue nuclear DAPI color, red membrane cytokeratin color and absence of green membrane CD 45 color.

Results

Cell images

Typical images of cells captured on a chip are seen in Figs. 5a-d, depicting 2 nucleated cells (blue); one a leukocyte (green) and the other an MDA-MB-231 cell (red). A composite image (Fig. 5d) shows all 3 colors.



Precision

Identical aliquots of cell-spiked blood samples were run repeatedly throughout one day and over the course of 3 days to evaluate the precision of the assay. Within day, day to day, and total CV's of the replicate samples were all <15% (Table 1).

Table 1 - Spiked cell precision

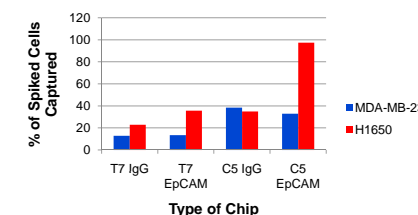
Day 1				Day 2				Day 3			
Sample	CTC #	Intra-sample CV	Inter-sample CV	Sample	CTC#	Intra-sample CV	Inter-sample CV	Sample	CTC#	Intra-sample CV	Inter-sample CV
1A	147			4A	173			7A	172		
1B	180			4B	151			7B	175		
1C	150			4C	178			7C	162		
1D	129	17.2		4D	162	6.8		7D	155	4.5	
2A	110			5A	157			8A	156		
2B	155			5B	161			8B	148		
2C	125			5C	140			8C	151		
2D	142	14.7		5D	139	7.3		8D	161	4.7	
3A	134			6A	168			9A	187		
3B	151			6B	196			9B	176	8.7	7.3
3C	136			6C	204						
3D	147	5	13.6	6D	209	8.9	12.4				

Total CV = 13.4%

Cell capture

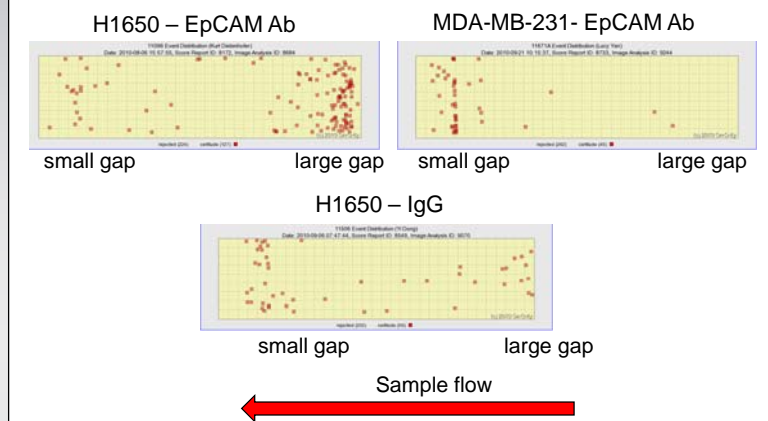
High (H1650) and low (MDA-MB-231) EpCAM expressing cells (data not shown) were spiked into blood from healthy donors and run on On-Q-ity T7 and gradient On-Q-ity C5 chips coated with either EpCAM Ab or normal mouse IgG (Fig. 6).

Fig. 6 - % cell capture by size or affinity



The distribution of H1650 and MDA cells across gradient On-Q-ity C5 chips (Fig. 7) demonstrates affinity capture across the chip for high EpCAM H1650 cells and size capture in the small gap sector for the low EpCAM MDA cells. As a control, H1650 cells run on a chip coated with an irrelevant IgG are localized almost exclusively in the small gap area, confirming size capture.

Fig. 7 – Cells run on On-Q-ity C5 chips with and without EpCAM Ab



Conclusions

1. On-Q-ity has developed an easy to use, microfluidic CTC platform that combines affinity and size capture with gradient C5 chips
2. Captured cells can be fluorescently detected on the chips as DAPI positive, cytokeratin positive, and CD 45 negative
3. The platform can capture spiked cells with good precision
4. High efficiency cell capture is possible only by combining size and affinity, with almost 100% efficiency of H1650 cells on the On-Q-ity C5 chip
5. Cells with high EpCAM expression are primarily captured across the chip surface by both size and affinity
6. Low EpCAM cells are primarily captured by size in the small gap sector
7. This dual size and affinity mechanism of the On-Q-ity CTC chip captures cancer cells with high efficiency

Bibliography

1. Nagrath S et al. Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature. 2007 Dec 20; 450(7173):1235-9.
2. Sequist LV et al. The CTC-chip: an exciting new tool to detect circulating tumor cells in lung cancer patients. J Thorac Oncol. 2009 Mar; 4(3):281-3.

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