Electronic Supplementary Material (ESI) for Lab on a Chip.

Lab on a Chip
Supplementary Information

Title: Stimulus responsive release of viable Circulating Tumor Cells (CTCs) following size-based capture

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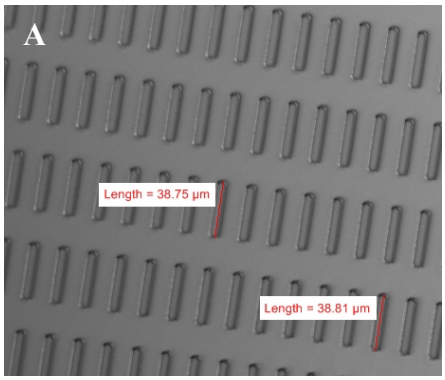
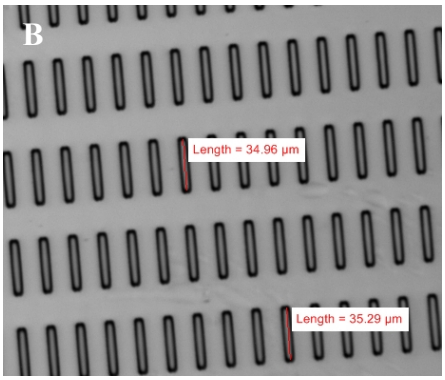
1. Calculation of slot filter parameters pre- and post- PIPAAm coating.

To measure the effect of PIPAAm coating on slot filter pore features, we measured the slot filter pre- and post-PIPAAm coating using phase contrast microscopy (Nikon Eclipse Ti-E). 3 filters pre- and post- coating was imaged. 20 images per filter were captured using using a 10X objective and the pore lengths and widths measured using the Nikon NIS-Elements Advanced Research software.



Slot filter before coating



Slot filter after coating

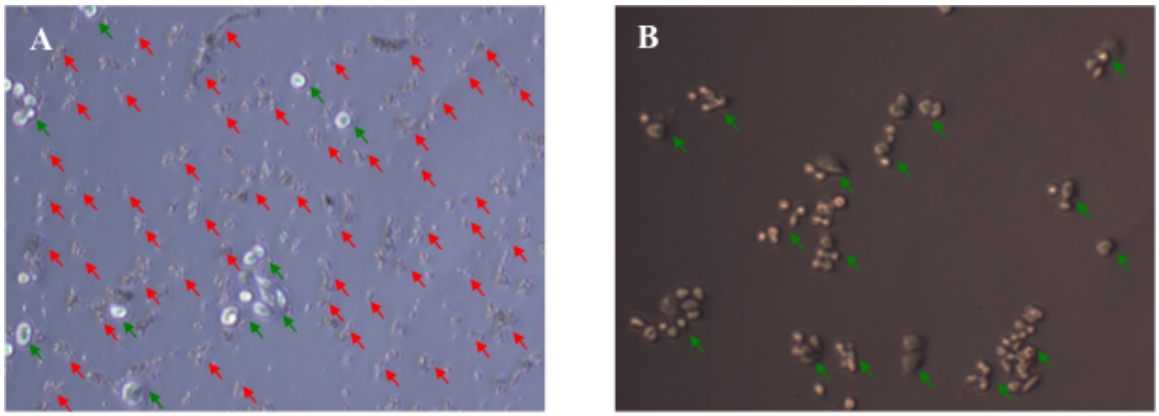
C

Filter	Average Length	Average Width
Non-coated	38.08±0.82	6.35±0.34
PIPAAm-coated	35.31±0.79	5.37±0.35
Decrease Percentage	7.3%±2.1%	15.3%±5.6%

Supplementary Figure 1 Measurement of pore parameters pre- and post- PIPAAm coating**. A)** Slot filter pre- and **B)** post- PIPAAm coating was imaged using Nikon Eclipse Ti-E microscope **C)** Pore length was found to be decreased by 7.3%±2.1% after PIPAAm coating and pore width was found to be decreased by 15.3%±5.6% after PIPAAm coating.

2. Removal of contaminating erythrocytes and leukocytes by gentle washes post-capture and release.

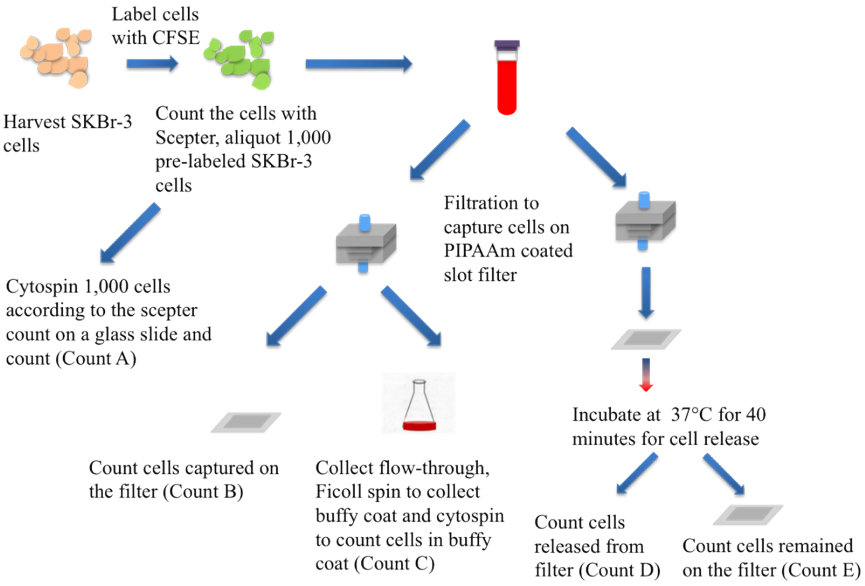
Since contaminating erythrocytes and leukocytes hamper the growth of released cells, we remove them by gentle washes at Day 1 in culture (16-24 hours post-release). Briefly, after target cells (SKBr-3 cells) fully attached to the culture plate, original culture medium was removed and replaced with fresh medium and gentle agitation was applied. Apoptotic erythrocytes and leukocytes were removed since they generally did not adhere to the culture plate. This process was repeated 1-2 times to ensure ~90% of the unwanted cells were removed.



Supplementary Figure 2 Contaminating erythrocytes and leukocytes were removed by gentle washes. ~1,000 SKBr-3 cells were retrieved from blood by PIPAAm coated slot filter and were plated on a 48-well plate. (A) At 16 hours in culture, SKBr-3 tumor cells adhered to culture plate (green arrows), whereas apoptotic erythrocytes and leukocytes were also settling at the bottom of the plate (red arrows). (B) Post-wash, non-adherent cells were removed, leaving adherent tumor cells on the plate (green arrows).

3. Calculation of tumor cell capture and release efficiency using PIPAAm coated slot filter

To test the efficiency of CTC capture and release using the PIPAAm coated slot filters, we first labeled SK-Br-3 breast cancer cells (ATCC, Manassas, VA) with green fluorescence using CellTrace CFSE Cell Proliferation Kit (Gibco, Life Technologies, Grand Island, NY). The experimental procedure is illustrated in details in Supplementary Fig. 1. Briefly, ~1,000 of the pre-labelled fluorescent SK-Br-3 cells, based on cell count using Scepter Automated Cell Counter (Millipore, Gibbstown, NJ), were spiked into 3 mL of healthy donor's blood. One third of the cell suspension was spun onto a glass slide for cell counting under fluorescence microscopy (Zeiss Axiovert 200M, Jena, Germany) to quantify and verify the number of cells in the blood **(Count A)**. The remaining spiked blood was then diluted 1:1 with 3 mL of Hank's Balanced Salt Solution (HBSS) (Gibco, Life Technologies, Grand Island, NY) and two equal aliquots of the cell suspension were processed in parallel through PIPAAm coated slot filters at a constant flow rate of 75 mL/hour. Post capture, 1 mL of sterile HBSS was filtered through at the same flow rate to remove and remaining red blood cells and debris. Next, one filter was directly mounted on a glass coverslip and examined under the fluorescence microscope to enumerate the number of cells captured on filter **(Count B)**. The flow-through of the sample was also examined for cell loss during filtration **(Count C)**. For the second sample, we reversed the filtration cassette and used 1 mL of McCoy's 5A culture medium (Gibco, Life Technologies, Grand Island, NY) to flush out cells trapped in the pores at a constant flow rate of 100 mL/hour. Post reverse-flow, the filter was then incubated in culture medium at 37°C (VWR symphony incubator, Radnor, PA) for 20 minutes. Post incubation, the release medium was spun onto the glass slide using a Statspin Cytofuge (Beckman Coulter, Miami, FL) for enumeration of the cells released from the filter **(Count D)**. The filter was examined, post cell release, to insure effective cell collection **(Count E)**



Supplementary Figure 3 Illustration of the experimental workflow to calculate tumor cell capture and release efficiency using PIPAAm coated slot filter.

	A	B	C	D	E	Capture Efficiency (B/A)	Release Efficiency (D/B)	Overall Retrieval Rate (D/A)
Replicate 1	787	798	5	614	175	101%	77%	78%
Replicate 2	1010	986	20	816	122	98%	83%	81%
Replicate 3	986	825	15	708	113	84%	86%	72%
Average						94%±9%	82%±5%	77%±5%

Supplementary Table 1. Calculation of CTC capture and release rates using pre-labelled SK-Br-3 cells spiked in healthy donor's blood as a model system. Three replicates were done on different dates, and calculations were done within each set of replicates.

	A	B	C	D	E	Capture Efficiency (B/$\bar{A}$)	Release Efficiency (D/B)	Overall Retrieval Rate (D/$\bar{A}$)
Replicate 1	864	907	27	757	152	95%	92%	80%
Replicate 2	1017	717	8	672	105	75%	81%	71%
Replicate 3	974	854	13	535	334	90%	65%	56%
Average	952	826	16	655	197	87%±10%	79%±14%	69%±12%

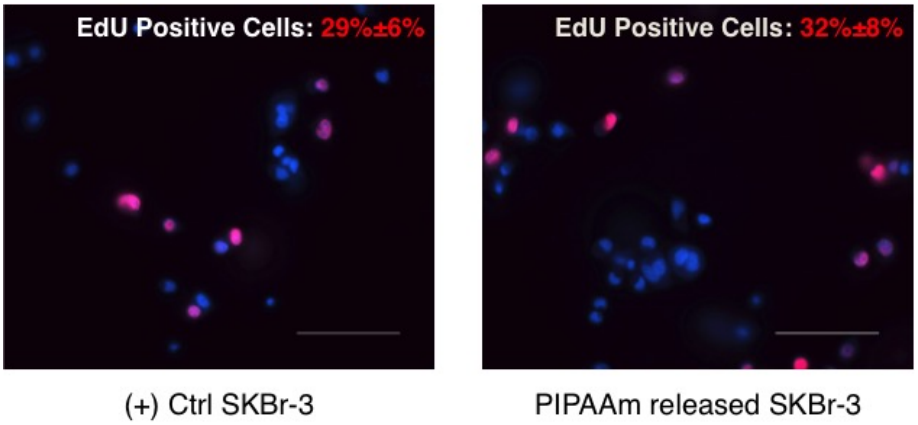
Supplementary Table 2. Calculation of capture and release rates with LMTS-GFP cells spiked in healthy donor's blood as a model system. Three replicates were done on the same date with the same aliquots, so calculations were done on averaged numbers with each replicate.

	A	B	D	Capture Efficiency (B/$\bar{A}$)	Release Efficiency (D/$\bar{B}$)	Overall Retrieval Rate (D/$\bar{A}$)
Replicate 1	1325	1234	89	93%	7%	7%
Replicate 2	1281	1179	77	89%	6%	6%
Replicate 3	1378	1151	69	87%	6%	5%
Average	1328	1188	78	89%±3%	7%±1%	6%±1%

Supplementary Table 3. Calculation of SKBr-3 capture and release rates using non-coated filters with SKBr-3 cells spiked in healthy donor's blood as a model system.

4. Measurement of proliferation rates of SKBr-3 tumor cells pre- and post- PIPAAm coated slot filter capture and release.

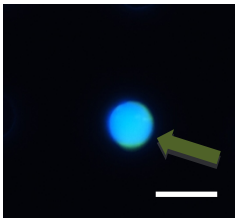
To test if the proliferation rate of tumor cells was altered by the PIPAAm coated slot filter capture and release process, we measured the proliferation rate of control SKBr-3 cells and PIPAAm released SKBr-3 cells using Click-iT® EdU Cell Proliferation Assays (Life Technologies, Grand Island, NY). All cells were labeled as Hoechst positive (Blue) whereas newly proliferated cells were also labeled as EdU-Alexa 594 positive (Red). Cells cultured on coverslips were incubated in the assay solution for 2 hours then stained as per the manufacturers protocol. Proliferation rates were calculated by percentage of newly proliferated cells (EdU labeled cells). Coverslips were imaged under a 40X objective and we enumerated cells in 10 fields on each coverslip. Each experiment was conducted in triplicate. No significant differences were observed (p-value = 0.44) between SKBr-3 cells plated as controls and SKBr-3 cells released from PIPAAm coated slot filter.

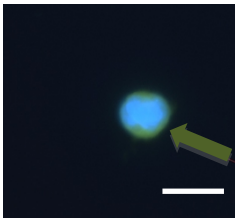


Supplementary Figure 4 Proliferation rates of SKBr-3 cells were compared before and after PIPAAm filter capture and release, as measured using EdU assay. (A) SKBr-3 cells plated as controls (B) SKBr-3 cells retrieved from blood by PIPAAm coated slot filters. Scale Bar: 100 μm

5. Test PIPAAm coated slot filter's functionality on clinical samples.

To validate that the PIPAAm coated slot filter can be applied to clinical samples, we collected 15 mL of blood from 4 metastatic breast cancer patients. Each sample was split into 2 samples of 7.5 mL. For one sample, the number of CTCs were analyzed using our well-validated round-pore filter. For the second sample, CTC capture and release using the PIPAAm coated slot-pore filter was assessed. The cells were then subject to immunofluorescence staining of Pan-cytokeratin-Alexa 488 and CD45-Alexa 680. CTCs were identified as DAPI+ CK+ and CD45- cells. CTC numbers were found comparable on round-pore filter and released from PIPAAm coated slot filters for all four clinical samples tested.





	CTC captured on round-pore filter	CTC released from PIPAAm coated slot filter
Patient 1	8	9
Patient 2	7	8
Patient 3	5	3
Patient 4	9	10

Supplementary Figure 5. (Top Panel) Enumeration of CTCs from metastatic breast cancer patients captured using round-pore filters or PIPAAm coated slot filters. Samples were subject to immunofluorescence staining for markers of Pan-cytokeratin (Alexa 488 -Green) and CD45 (Alexa 680 – White). The sample was then counter-stained with DAPI (Blue). CTCs were identified as DAPI+ CK+ CD45- cells. Scale Bar 20 μm **(Bottom Panel)** CTC enumeration and release using round-pore filters and PIPAAm coated slot pore filters.