

Rapid and Efficient Isolation of Circulating Tumor Cells (CTCs) from Whole Blood Using Lithographically Fabricated Microfilters

Daniel Adams¹, Olga Makarova², Peixuan Zhu¹, Shuhong Li¹, Platte T. Amstutz³, Cha-Mei Tang³¹ Creatv MicroTech, Inc., Rockville, MD, ² Creatv MicroTech, Inc., Chicago, IL, ³ Creatv MicroTech, Inc., Potomac, MD

ABSTRACT

We have developed lithographically fabricated microfilters with precision pore dimensions and high porosity, able to rapidly capture CTCs from whole blood. Breast cancer cell lines, MCF-7, SKBR3 and MB453, were used to evaluate the microfilters performance. Enumerated cells were spiked into 7.5 mL whole human blood and by negative pressure. The cells captured on the filter were fixed, permeabilized, and stained for DAPI, cytokeratin (FITC), and EpCAM (TRITC) while showing no CD45 marker (CY5). Contaminating blood cells were identified by the presence of CD45 with DAPI. There was no observable contamination by red blood cells, while white blood cells average about 2500 on the microfilter. The assay was completed in less than 2 hours, with capture efficiencies of $97 \pm 4\%$, $100 \pm 2\%$, and $94 \pm 5\%$, for MCF-7, SKBR-3 and MB453 cell lines, respectively. Microfiltration and immuno-identification of CTCs on high porosity, precision microfilter is simple, rapid and efficient.

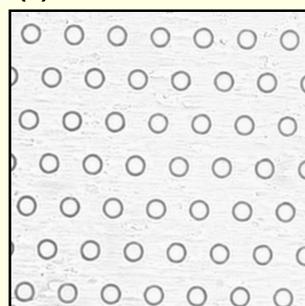
INTRODUCTION

CTCs are cancer cells disseminated from primary, or metastatic tumors, which can be used to monitor therapy response and predict disease outcome.¹ Isolation of CTCs by size exclusion is a technique with the advantage of capturing cells without reliance on surface marker expression.¹⁻⁴ For many years CTC filtration technology has relied on track-etch microfilters, which have randomly located pores that can overlap producing an effectively larger pores and low-porosity (Figure 1b).

In contrast, we have developed CellSieve™ microfilters with precision pores and high porosity (Fig. 1a) that are more suitable for CTC isolation. They are strong, transparent, biocompatible, and non-fluorescent with 8 μm diameter pores patterned in a uniform array with approximately 160,000 pores within a 9 mm diameter area in a 13 mm filter format (Figure 1a).

Microfiltration using CellSieve™ microfilters is a simple method for the isolation of CTCs from large volumes of whole human blood. Filtration can be performed in less than 2 minutes with consistently high capture efficiency and low blood cell contamination.

(a) CellSieve™ Filters



(b) Track-etch Filters

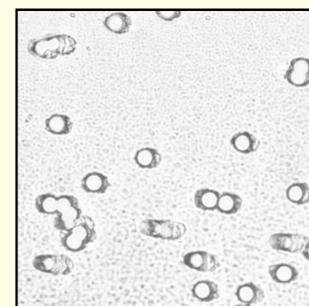


Figure 1. (a) CellSieve™ microfilter fabricated by lithography method with regularly distributed pores, and (b) track-etch filter with randomly overlapping distributed pores.

MATERIALS & METHODS

We describe an assay to capture and enumerate CTCs spiked into whole human blood using CellSieve™ microfilters. MCF-7, SKBR3 and MB453 human adenocarcinoma cell lines (ATCC) were used to evaluate the assay performance.

Cells were first enumerated to obtain precise input, then spiked into 7.5 mL whole human blood, mixed with 7.5 mL fixative buffer, and placed into a syringe. Filtration was performed using a microfilter mounted in a filter holder, with the sample drawn by negative pressure at a flow rate of ~10 mL/min. Cells captured on the filter were fixed a second time,

permeabilized, and blocked inside the filter holder apparatus. Cells were then stained by antibodies against cytokeratins 8, 18, & 19 conjugated to FITC, EpCAM conjugated to phycoerythrin, and CD45 conjugated to Cy5. Following a wash, the microfilter was removed, mounted onto a microscope slide, and stained with DAPI. CTCs were identified by the presence of DAPI, cytokeratin, and EpCAM, while showing no CD45 marker. Contaminating blood cells were identified by the presence of CD45, and/or DAPI cells without EpCAM and cytokeratin. The above experiments were performed in triplicate.

RESULTS

Consistent with previously presented data, the CTC capture efficiency averaged 97% (Table 1). Total assay time, from capture through staining, was less than 2 hours. In addition to high capture efficiency, we demonstrated that CTC isolation and immuno-staining of EpCAM cytokeratin 8,18, & 19 and CD45 can be performed rapidly and easily on the CellSieve™ microfilter. The fluorescence imaging was clear and simple to interpret as the background fluorescence was negligible (Figure 2).

Contamination by red blood cells, which are easily visualized under white light, was not observed. Other blood cell contamination fell into two groups: cells that were only DAPI positive, averaging 2929 cells per filter, and cells that were both DAPI positive and CD45 presenting, averaging 2583 cells per filter.

Figure 2. Individual and merged fluorescent images of stained MCF-7 cell and white blood cells on CellSieve™ microfilter: (a) Overlay image, (b) DAPI filter for nucleus, (c) FITC filter for CK 8, 18, 19, (d) TRITC filter for EpCAM, and (e) Cy5 filter for CD45.

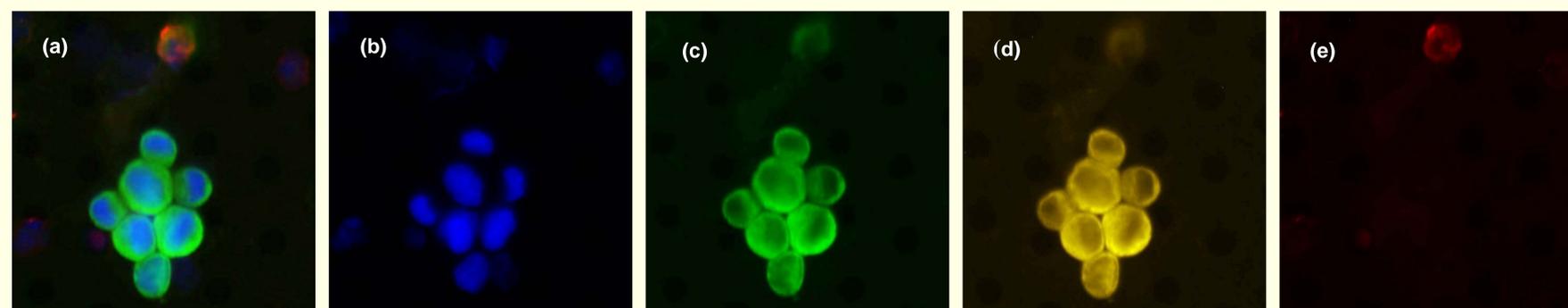


Table 1. Recovery of CTCs on CellSieve™ Microfilters

Cell Type	Input Cell Count	Cell Count on Filter	% Recovery on Filter
MCF-7	101	99	98%
	89	89	100%
	28	26	93%
Average			97±4%
SKBR-3	40	40	100%
	50	49	98%
	39	40	103%
Average			100±2%
MDA-MB-453	56	51	91%
	32	29	91%
	40	40	100%
Average			94±5%

CONCLUSIONS

- Filtration of whole blood using CellSieve™ takes < 2 min.
- CTC isolation and immuno-staining can be performed in < 2 hours
- CellSieve™ can capture $97 \pm 4\%$ of cells from whole human blood
- Red blood cell contamination is not observed.
- White blood cells on CellSieve™ microfilter average 1250-3900 per 7.5 mL sample or 0.000001% of input.

References

1. Vona, G, *et al.* (2000). "Isolation by Size of Epithelial Tumor Cells A New Method for the Immunomorphological and Molecular Characterization of Circulating Tumor Cells." *American Journal of Pathology* **156**(1): 57-63.
2. Zheng, S., *et al.* (2007). "Membrane microfilter device for selective capture, electrolysis and genomic analysis of human circulating tumor cells." *J. Chromatogr. A*. **1162**:154-61.
3. Hosokawa, M, *et al.* (2010). "Size-Selective Microcavity Array for Rapid and Efficient Detection of Circulating Tumor Cells." *Anal. Chem.* **82**: 6629-6635.
4. Farace, F, *et al.* (2011). "A direct comparison of CellSerach and ISET for circulating tumor-cell detection in patients with metastatic carcinomas." *BJC advance online publication* **9 August 2011**: 1-7.